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Study on Proline-specific Aminopeptidases and Their Application on Food Processing

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Research aims

Hydrolyzed vegetable proteins produced through hydrochloric acid hydrolysis (acid-HVP) are widely used as the savoury ingredient and have been added to many food. Recently, it became a serious problem because acid-HVP is contaminated with a group of chemicals known as chloropropanols which include 1, 3-dichloropropanol (1,3-DCP) and 3-monochloropropane-1, 2-diol (3-MCPD). These compounds are by-products in acid-HVP and have insalutary effects on human body. During the acid hydrolysis, components of fats and oils in the starting materials may be chlorinated at high temperature to form chloropropanols. In contrast, enzymatic hydrolysis of proteins produces no such compounds and therefore it is considered safe. Proteases have been utilized for food processing for long time and there are a large number of basic researches on the enzymatic hydrolysis of proteins or peptides into specific peptides. However, the enzymatic method cannot digest proteins completely into amino acids and has not yet been applied as a practical method. This is due to the fact that ordinary peptidases cannot or hardly act on peptide bonds around proline residues. Our main purpose here is to understand the substrate recognition and hydrolysis mechanism of the proline-specific aminopeptidases in order to develop a practical enzymatic method to achieve a complete digestion of proteins.

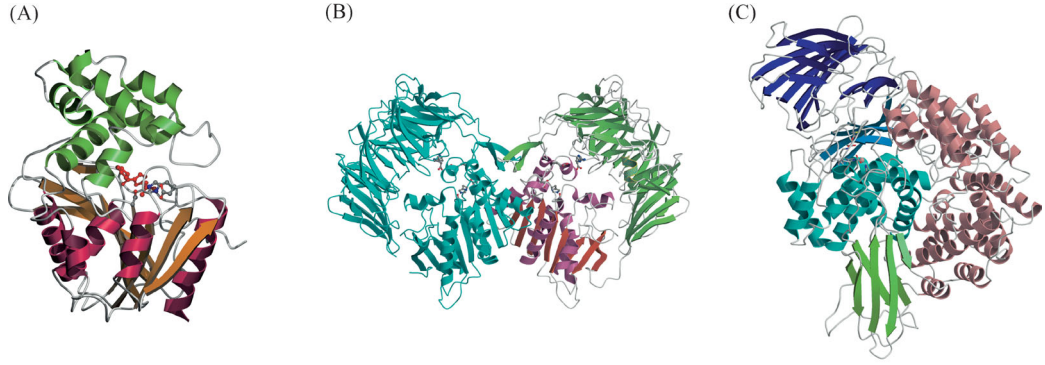
Methods

Prolyl aminopeptidase (PAP) from *Serratia marcescens*, prolyl tripeptidyl aminopeptidase (PTP) from *Porphyromonas gingivalis*, and aminopeptidase N (APN), which can release a N-terminal proline residue of the substrate peptide in *Escherichia coli*, were used as proline-specific aminopeptidases. Wild-type forms of PAP[1] and APN[2] were expressed in *E. coli*. N-terminal truncated form of PTP (PTPΔ39) lacking the membrane-anchoring region was expressed as a soluble form of enzyme[3]. Enzymes were purified to homogeneity and crystallized by using a hanging drop vapor diffusion method. The enzyme structures were determined by X-ray crystallography using data collected at synchrotron facilities[4–6].

Results

(1) Prolyl aminopeptidase: The presence of a hydrophobic pocket that recognizes the N-terminal proline residue was clarified from the crystal structures of the PAP-inhibitor complexes. Interestingly, there was an unusual extra space at the bottom of the hydrophobic pocket where proline residue is fixed in the PAP. Moreover, the 4-acetyloxyl group of the substrate can be accommodated in this space.

(2) Prolyl tripeptidyl aminopeptidase: PTP was a dimmer and the each monomer is consisted of β-propellar and catalytic domains, and a large cavity between the domains. Those structural features are common in the prolyl oligopeptidase (POP) family. The catalytic triad was conserved in the catalytic domain and a proline-recognizing hydrophobic pocket was identified. The PTP structure is similar to dipeptidyl aminopeptidase IV (DPIV), which recognizes a proline residue at the penultimate position from the N-terminus and releases a dipeptide. However, there were characteristic differences which were related to the substrate specificity of tripeptidyl activity. The N-terminal amino group was recognized by two carboxyl groups of Glu205 and Glu206 from a helix in dipeptidyl aminopeptidase IV. In prolyl tripeptidyl aminopeptidase, however, the



Structures of the three kinds of aminopeptidases. (A) PAP, (B) PTP, and (C) APN.

Glu205 (located in the loop) and Glu636 were found to carry out this function. The loop structure provides sufficient space to accommodate three N-terminal residues (Xaa-Xaa-Pro) of substrates.

(3) Aminopeptidase N: APN was composed of four domains: an N-terminal β-domain, a catalytc domain, a middle β-domain, and a C-terminal α-domain. The structure of the catalytic domain exhibits similarity to thermolysin. The groove on the catalytic domain that contains the active site is covered by the C-terminal α-domain, and a large cavity is formed inside the protein. However, there existed a small hole at the center of the C-terminal α-domain. The amino terminus of bestatin was recognized by Glu121 and Glu264. Glu298 and Tyr381 are considered to be involved in peptide cleavage. A difference revealed between the ligand-free form and the enzyme-bestatin complex indicated that Met260 functions as a cushion to accept substrates with different amino terminal sizes, resulting in the broad substrate specificity of this enzyme.

Conclusion

We have clarified the crystal structures of two proline-specific aminopeptidases and of the aminopeptidase N which shows a broad substrate specificity including a proline residue. Based on these structures, proline recognition mechanism can be discussed from the view of three dimensional structure. These findings may be able to overcome the difficulty that ordinary peptidases can not or hardly act on peptide bonds around proline residues and will afford insights into a base in developing a practical and efficient enzymatic method to digest proteins completely into amino acids.

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