Structure-Function Studies on Prolyl Oligopeptidase

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Prolyl oligopeptidase hydrolyzes the peptide bond on the C-terminal side of proline in peptides up to around 30 amino acid residues. The enzyme is involved in hormone and neuropeptide processing and is implicated in amnesia, depression and blood pressure regulation. Crystal structure determination of porcine prolyl oligopeptidase revealed a two domain structure in which the catalytic domain has an alpha-beta hydrolase topology similar to lipases and esterases [1]. The Ser-Asp-His catalytic triad is covered by the central tunnel of an unusual seven-bladed open-topology beta propeller domain which lacks a molecular 'velcro' between the first and last blades. Oscillation of the propeller blades serves as a gating filter that excludes large structured peptides, protecting cytosolic proteins from proteolysis [2]. The mechanisms of catalysis and regulation were further investigated using a combination of site-directed mutagenesis, kinetic measurements, X-ray crystallography and synthetic peptide chemistry. Enzyme variants containing engineered disulphide bridges demonstrated the requirement for concerted movements of the peptidase and propeller domains in addition to separation of the propeller blades during substrate entry. Substrate peptides displaying different kinetic profiles were shown to form similar enzyme-substrate complexes, highlighting the importance of enzyme-substrate interactions that occur on route to the Michaelis complex [3]. Variants lacking the catalytic aspartic acid revealed the catalytic importance of this residue. However, it is not required to stabilize the catalytically competent position or tautomer of the catalytic histidine [4]. A variant with a deficient oxyanion binding site demonstrated the importance of electrophilic catalysis for oxyanion stabilization [5]. The catalytic contributions of the catalytic aspartic acid and the oxyanion binding site are more important during hydrolysis of a peptide with a stronger scissile bond.

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