An investigation on catalysis of acylaminoacyl peptidases

PhD Thesis

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1. Introduction

Serine peptidases contain two residues at the active site in addition to the catalytic triad (His, Asp, Ser), which form a cavity called “oxyanion hole” that accommodates the negatively charged oxyanion in the transition state of the catalysis and donate two H-bonds.

Enzymes of the prolyl oligopeptidase (POP) family (acylaminoacyl peptidase, prolyl oligopeptidase, dipeptidyl peptidase IV and oligopeptidase B) are extensively studied. These enzymes are larger than classical serine peptidases and are composed of a peptidase domain with an α/β hydrolase fold and a β-propeller domain. POP and oligopeptidase B are monomeric enzymes and endopeptidases, while the exopeptidase acylaminoacyl peptidase and dipeptidyl peptidase IV are tetrameric and dimeric enzymes, respectively.

Acylaminoacyl peptidase (AAP) cleaves acylated amino acids from the N-terminus of the N-acylated peptides that plays important role in many biological and disease processes. Human AAP is encoded by the DNF15S2 locus on the short arm of chromosome 3 at the region 21, which suffers deletions in small cell lung carcinomas, and renal carcinomas, resulting in deficiency in the expression of the enzyme. Acylaminoacyl peptidase is also supposed to be involved in the degradation of oxidatively damaged proteins in cells and can be associated with various diseases where damaged proteins aggregate. Its involvement in cataract formation and in the breakdown of immunogenic formylmethionyl-peptides in the digestive tract after bacterial attack was also suggested.

This work reveals the structural grounds of the oxyanion binding site of AAPs and determines novel substrate specificity of the archeal prototype of AAP. Enzyme kinetic studies and X-ray structures highlight the evolutional change in the substrate specificity of AAPs.
2. Methods used

Standard molecular biological techniques (PCR, agarose gel electrophoresis, DNA purification and cloning) were applied to obtain modified gene constructs. Recombinant proteins were expressed in *E. coli* strains and purified with various chromatographic techniques. Kinetic measurements were carried out using photo- and fluorometric substrates, respectively and the signals were detected with a Cary-100 spectrophotometer and a Cary Eclipse fluorescent spectrophotometer, respectively. The physical stability of the enzyme variants were analyzed with DSC (differential scanning calorimetry) and photo-absorption techniques. Enzyme-inhibitor and enzyme-substrate complexes were crystallized applying the “hanging drop” method and structures were solved with X-ray crystallography.

3. Results

The His507 residue of the acylaminoacyl peptidase from porcine liver (AAP) was found to be a potential residue of the oxyanion binding site. Changing this amino acid to Ala resulted in about 100 fold reduction of the $k_{cat}/K_m$ for the hydrolysis of acetyl-Ala-4-nitroanilide substrate. His507 is situated in the "HGGP" motif that is conserved in acylaminoacyl peptidases. Its participation in the catalysis was further studied with the *Aeropyrum pernix* K1 acylaminoacyl peptidase (apAAP) with a known three dimensional structure. The corresponding histidine residue in apAAP is the His367. We mutated His367 to alanine, and the variant enzyme was studied in kinetic assays and crystallised. We determined its X-ray structure in collaboration with Veronika Harmat at the protein modelling group of ELTE. The X-ray crystallography of the His367Ala variant (pdb: 2qr5) revealed that the imidazole ring of His367 stabilizes the spatial orientation of the catalytically competent Gly369, one of the two members of the oxyanion hole.

The AAP cleaves N-acylated amino acids from the N-terminus of acylated peptides. Our structures of apAAP complexed with product-like inhibitors, Ac-Phe (pdb: 2hu7) and Gly-Phe (pdb: 2hu5) revealed that the substrate binding site of apAAP extends beyond
the S2 subsite thus may be capable of binding peptides with longer N-terminuses. This endoproteolytic activity was demonstrated by determining the cleavage patterns of various oligopeptide substrates. The heptapeptide Abz-GFEPF(NO_2)RA substrate was co-crystallized with the catalytically inactive (Ser445Ala) variant of apAAP. Unfortunately, the substrate was cleaved under the lengthy crystallization period and only the acyl portion (Abz-GF-OH) was seen in the X-ray structure (pdb: 2hu8). However, the Edman degradation of a partial hydrolysis product of the peptide substrate Abz-KARVLF(NO_2)EANle confirmed the endopeptidase activity of apAAP, as the cleavage occurred predominantly after F(NO_2), which is the 7th residue from the N-terminus.

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4. Publications

