アスパラギン酸405残基は アミノペプチダーゼBの基質特異性に寄与する

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Aspartic Acid 405 Contributes to the Substrate Specificity of Aminopeptidase B

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ABSTRACT : Aminopeptidase B (EC 3.4.11.6, ApB) specifically cleaves *in Vitro* the *N*-terminal Arg or Lys residue from peptides and synthetic derivatives. Ap B was shown to have a consensus sequence found in the metallopeptidase family. We determined the putative zinc binding residues (His324, His328, and Glu347) and the essential Glu325 residue for the enzyme using site-directed mutagenesis (Fukasawa, K. M., et al. (1999) *Biochem. J.* 339, 497-502). To identify the residues binding to the amino-terminal basic amino acid of the substrate, rat cDNA encoding ApB was cloned into pGEX-4T-3 so that recombinant protein was expressed as a GST fusion protein. Twelve acidic amino acid residues (Glu or Asp) in ApB were replaced with a Gln or Asn using site-directed mutagenesis. These mutants were isolated to characterize the kinetic parameters of enzyme activity toward Arg-NA and compare them to those of the wild-type ApB. The catalytic efficiency (k_{cat}/K_m) of the mutant D405N was 1.7 x 10^4 M^{-1} s^{-1}, markedly decreased compared with that of the wild-type ApB (6.2 x 10^5 M^{-1} s^{-1}). The replacement of Asp405 with an Asn residue resulted in the change of substrate specificity such that the specific activity of the mutant D405N toward Lys-NA was twice that toward Arg-NA (in the case of wild-type ApB; 0.4). Moreover, when Asp405 was replaced with an Ala residue, the k_{cat}/K_m ratio was 1000-fold lower than that of the wild-type ApB for hydrolysis of Arg-NA; in contrast, in the hydrolysis of Tyr-NA, the k_{cat}/K_m ratios of the wildtype (1.1 x 10^4 M^{-1} $s^{-1}$and the mutated (8.2 x 10^3 M^{-1} s^{-1}) enzymes were similar. Furthermore, the replacement of Asp-405 with a Glu residue led to the reduction of the k_{cat}/K_m ratio for the hydrolysis of Arg-NA by a factor of 6 and an increase of that for the hydrolysis of Lys-NA. Then the k_{cat}/K_m ratio of the D405E mutant for the hydrolysis of Lys-NA was higher than that for the hydrolysis of Arg-NA as opposed to that of wild-type ApB. These data strongly suggest that the Asp 405 residue is involved in substrate binding via an interaction with the P1 amino group of the substrate's side chain.

抄録　アミノペプチターゼBの基質特異性は、405番残基のアスパラギン酸が関与していることを野生型と種種の変異型の触媒効率を比較することで明らかにした。

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