Study of Secondary Specificity of Enteropeptidase in Comparison with Trypsin

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Abstract—A comparative study of secondary specificities of enteropeptidase and trypsin was performed using peptide substrates with general formula A-(Asp/Glu)$_n$-Lys(Arg)-↓-B, where n = 1-4. This was the first study to demonstrate that, similar to other serine proteases, enteropeptidase has an extended secondary binding site interacting with 6-7 amino acid residues surrounding the peptide bond to be hydrolyzed. However, in the case of typical enteropeptidase substrates containing four negatively charged Asp/Glu residues at positions P2-P5, electrostatic interaction between these residues and the secondary site Lys99 of the enteropeptidase light chain is the main factor that determines hydrolysis efficiency. The secondary specificity of enteropeptidase differs from the secondary specificity of trypsin. The chromophoric synthetic enteropeptidase substrate G_5DK-F(NO_2)G (k_{cat}/K_m = 2380 $mM^{-1}\cdot min^{-1}$) is more efficient than the fusion protein $PrAD_4K$-P26 (k_{cat}/K_m = 1260 $mM^{-1}\cdot min^{-1}$).