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Glutamyl endopeptidase of bacillus intermedius, strain 3-19. purification, properties, cristallization

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Abstract

A homogeneous glutamyl endopeptidase, splitting peptide bonds of glutamic. rarely - of aspartic acid residues in peptides and proteins, was isolated from Bacillus intermedius 319 culture filtrate using chromatography on CM cellulose and Mono S. The enzyme molecular mass =29kDa. pI 8.4. The pro teinase is inhibited by I)FP. The enzyme, like other glutamyl endopeptidasos, reveals two pl[optima at pH 7.5 and 9.0 for casein and one - at pH 8.0 for Z-Glu-pNA hydrolysis. K =6 mM was found for hydrolysis of the lat ter substrate. Its activity optimum lies at 55(;. The enzyme is stable at ptf 6.5-11.0. Its N-erminal sequence shows 56 per cent coinciding residues. when compared with that of Bacillus licheniformis glutamyl endopeptidase: VVIGDI) GRTKVA'NTRVAPYXXXXITFGGS-. The crystal prismesor plates of the size 0.23-0.3 x 0.15-0.2 x 0.07-0.1 mm have been grown using the vapour diffusion technique in lhe hanging drop followed by macroseeding. The crystals belong to the spat(group B2 with following unit cell parameters: a=69.59 angstrom; b=61.613 angstrom; c=56.107 angst rom; ,-117.57. The x-ray data set to 1.7 angstrom resolution was collected on the synchrotron (EMBL Gain burg).