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Isolation and characterization of glutamyl endopeptidase 2 from Bacillus intermedius 3-19

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Abstract

The culture filtrate of Bacillus intermedius 3-19 was used for isolation by chromatography on CM-cellulose and Mono S columns of a proteinase that is secreted during the late stages of growth. The enzyme is irreversibly inhibited by the inhibitor of serine proteinases diisopropyl fluorophosphate, has two pH optima (7.2 and 9.5) for casein hydrolysis and one at pH 8.5 for Z-Glu-pNA hydrolysis. The molecular weight of the enzyme is 26.5 kD. The Km for Z-Glu-pNA hydrolysis is 0.5 mM. The temperature and pH dependences of the stability of the proteinase were studied. The enzyme was identified as glutamyl endopeptidase 2. The N-terminal sequence (10 residues) and amino acid composition of the enzyme were determined. The enzyme hydrolyzes Glu4-Gln5, Glu17-Asp18, and Cys11-Ser12 bonds in the oxidized A-chain of insulin and Glu13-Ala14, Glu21-Arg22, Cys7-Gly8, and Cys19-Gly20 bonds in the oxidized B-chain of insulin.

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Keywords

Bacillus intermedius, Glutamyl endopeptidase, Isolation, Properties, Proteinase