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Purification and characterization of serine proteinase 2 from Bacillus intermedius 3-19

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Abstract

A proteinase secreted in the late stationary phase was isolated from the culture fluid of Bacillus intermedius 3-19 by ion-exchange chromatography on CM-cellulose followed by FPLC on a Mono S column. The enzyme was completely inhibited by the serine proteinase inhibitors diisopropyl fluorophosphate and phenylmethylsulfonyl fluoride. The maximum proteolytic activity against the synthetic chromogenic substrate Z-Ala-Ala-Leu-pNA was observed at pH 9.0. The molecular weight of the enzyme is 28 kD and its isoelectric point is 9.2. We have also determined pH- and thermostability and Km and k cat of this proteinase. The enzyme has been classified as a thiol-dependent serine proteinase. N-Terminal amino acid sequence (10 residues) and amino acid composition of the protein were also determined. By the mode of hydrolysis of peptide bonds in the oxidized B-chain of insulin, this enzyme is similar to the thiol-dependent serine proteinase 1 from B. intermedius 3-19 secreted during vegetative growth.

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Keywords

Properties, Proteinase, Purification, Thiol-dependent serine proteinase (Bacillus intermedius)