Glutamyl endopeptidase of *Bacillus intermedius*, strain 3-19

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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* 3-19 culture filtrate using chromatography on CM cellulose and Mono S. The enzyme molecular mass is equal to 29 kDa, pI 8.4. The protease is inhibited by diisopropylfluorophosphate. The enzyme, like other glutamyl endopeptidases, reveals two pH optima at pH 7.5 and 9.0 for casein and one at pH 8.0 for Z-Glu-pNA hydrolysis. A 6 mM K_m is found for hydrolysis of the latter substrate. The enzyme activity optimum lies at 55°C, and it is stable at pH 6.5–11.0. Its N-terminal sequence shows 56% coinciding residues when compared with that of *Bacillus licheniformis* glutamyl endopeptidase.

Key words: Protease; Glutamyl endopeptidase; *Bacillus intermedius*; Isolation; Specificity

1. Introduction

Glu,Asp-specific proteases (glutamyl endopeptidases) that split specifically the peptide bonds formed by α-carboxyl groups of glutamic and — to a lesser extent — of aspartic acid belong to a subfamily of proteolytic enzymes. Initially, a protease endowed with this specificity pattern was isolated from *Staphylococcus aureus* V8 [1,2], then from *Staphylococcus aureus* 92gn [3], *Actinomyces* sp. [4], *Streptomyces thermovulgaris* [5,6], *Streptomyces griseus* [7], *Streptomyces fradiae* [8], two strains of *Bacillus licheniformis* [9,10].

The enzymes have been referred to serine proteases, but only scarce information has been collected on their structural organization. According to X-ray crystallography data, their tertiary structure seems to be related to that common for chymotrypsin family [11]. It was suggested that an unusual array of three histidine residues in *Streptomyces griseus* protease is responsible for its specific interaction with γ-carboxylate of the substrate glutamyl residue [12]; albeit, no homologous, His residues were detected in other glutamyl endopeptidases.

Thus, further research is necessary to characterize this subfamily of proteolytic enzymes. The present paper reports the isolation and characterization of glutamyl endopeptidase from *Bacillus intermedius* strain 3-19.

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Abbreviations: DFP, diisopropylfluorophosphate; pNA, *p*-nitroanilide; Z, benzyloxycarbomyl; All amino acids are of the L configuration

2. Materials and methods

2.1. Bacterial strain

Streptomycin-resistant strain *Bacillus intermedius* 3-19 was obtained from culture collection of Kazan State University microbiological department. The cells were grown as described earlier [13].

2.2. Reagents

CM cellulose was purchased from Reanal (Hungary), and Mono S HR 5/5 FPLC and Sephadex G-25 from Pharmacia (Sweden). Servalyte and glucagon were from Serva (Germany).

2.3. Proteolytic activity assessment

Casein solution (2%) in 0.1 M Tris-HCl buffer (pH 9.0), or Z-Glu-pNA [14], were used as substrates. The activity unit was defined as the amount of the enzyme capable to produce 1 μmol of *p*-nitroanaline (pNA) per min from Z-Glu-pNA under the specific conditions [3]. The value of K_m was determined using 0.2–3.3 mM Z-Glu-pNA.

2.4. Isolation of the enzyme

Sodium acetate buffers (pH 6.3) were used throughout the procedure. The protease was purified from 2 l of the culture filtrate by ion-exchange chromatography on CM cellulose column (4.5×3 cm), equilibrated and washed with 0.02 M buffer. The protease was eluted with 0.2 M buffer, then rechromatographed on CM cellulose column (1×4 cm). The enzyme was eluted with 0.15 M buffer, the eluate diluted 10 times and applied to Mono S HR 5/5 FPLC column, equilibrated with

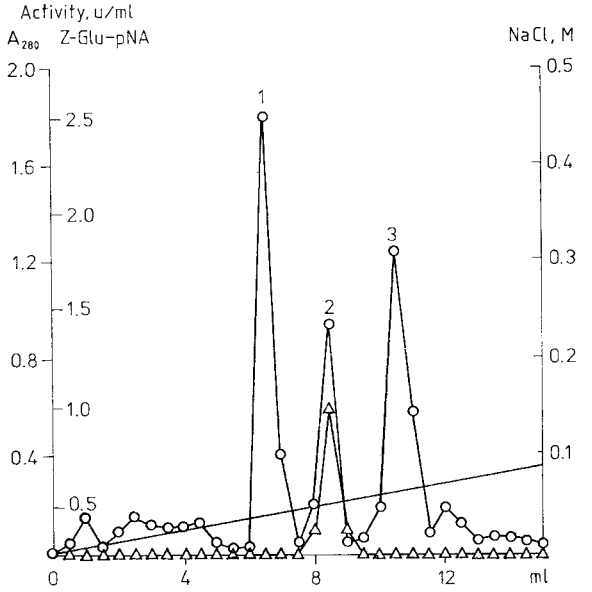

Fig. 1. Chromatography of *B. intermedius* glutamyl endopeptidase on Mono S. The mixture of enzymes obtained by CM cellulose chromatography was applied on Mono S HR 5/5 column. The enzymes were eluted with NaCl (0–0.5 M) concentration gradient in 15 mM acetate buffer (pH 6.3) containing 0.5 mM $CaCl_2$. ○, A_{280}; △, activity with Z-Glu-pNA. Fraction 2 contained pure glutamyl endopeptidase; fractions 1 and 3 contained subtilisin-like proteases to be described elsewhere.

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