PRIMARY STRUCTURE OF RIBONUCLEASE FROM *BACILLUS INTERMEDIUS* 7P

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1. Introduction

Studies on the relation between the structure and function of RNases have been carried out mainly with RNase A [1] and to a lesser extent with RNase T_1 [2]. Bacterial RNases have been significantly less studied in this respect. Up to now, the primary structure and some physico-chemical properties of only one bacterial RNase, namely the RNase of *B. amyloliquefaciens*, have been established [3–5].

The purpose of this study was to determine the primary structure of the extracellular RNase of *Bacillus intermedius*, strain 7P. The RNase catalyses the breakdown of RNA to oligonucleotides terminating in 3′-phosphate and is mainly specific towards the purine bases of the RNA molecule [6]. Nucleoside-2′·3′-phosphates and dinucleoside phosphates are completely resistant to the action of this enzyme. The large scale preparation of the homogeneous RNase and some characteristics of this enzyme were described in [7].

2. Materials and methods

The amino acid composition of the protein and peptides was determined using a Bio-Cal BC-201 amino acid analyser (GFR). The amino acid sequence of the N-terminal part of the protein was established on a Beckman 890C automatic sequenator. PTH derivatives of the amino acids were determined by thin-layer chromatography on polyamide plates [8] with subsequent scanning on a PMQ-II spectrophotometer (Opton, GFR) and processing of results according to [9], by gas-liquid chromatography [10], and by amino acid analysis after hydrolysis of PTH derivatives of amino acids with 57% HI [11].

The sequence of peptides was determined by the dansyl-Edman technique and using carboxypeptidases A, B and C and leucine aminopeptidase. The kinetics of the exopeptidase degradation of peptides were studied by means of the quantitative method of amino acid analysis based on their dansylation, followed by thin-layer chromatography on polyamide plates [12]. DNS-amino acids were scanned using an automatic system with a Saratov minicomputer [13].

Hydrolysis of the protein with the protease from the V8 strain of *Staphylococcus aureus* was performed in 0.1 M NH_4HCO_3 (pH 8.0) at 37°C for 15 h [14] at an enzyme–substrate ratio 1·30. Cleavage of the RNase tryptophanyl bonds was carried out with *N*-chlorosuccinimide according to [15].

3. Results and discussion

RNase of *B. intermedius*, strain 7P, has mol. wt ~12 300 and contains 109 amino acid residues [7].

The native protein was examined by automatic phenyl isothiocyanate degradation, and 40 out of the first 50 residues were unequivocally identified. The remaining residues were identified upon analysis of the peptides.

The mixture of peptides obtained after hydrolysis of RNase with *Staphylococcus aureus* protease was fractionated by gel filtration on a Sephadex G-50 (fine) column in 10% acetic acid, containing 8 M urea. The resulting fractions were further purified by ion-exchange chromatography on an SP-Sephadex