

Primary structure and catalytic properties of extracellular ribonuclease of *Bacillus circulans*

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A complete amino acid sequence of extracellular *Bacillus circulans* RNase was established and compared with a structure of *B. amyloliquefaciens* RNase. Gln¹⁵, Gly⁶⁵ and Gln¹⁰⁴ in *B. amyloliquefaciens* RNase were found to be replaced by Leu, Ala and Lys, respectively, in *B. circulans* RNase. Catalytic properties of *B. circulans* RNase were studied.

RNase; *Bacillus circulans*; Sequence; Catalytic property

1. INTRODUCTION

Extracellular microbial RNases form homologous protein family which consists of at least three subgroups, formed by the *Bacillus*, *Streptomyces* and fungal enzymes [1]. The most studied extracellular *Bacillus* RNases are RNase of *B. amyloliquefaciens* (RNase Ba), RNase of *B. intermedius* (RNase Bi) and RNase of *B. thuringiensis* (RNase Bth). The amino acid sequences of these RNases have been established [2–4] and three-dimensional structures of RNases Ba and Bi have been determined [5]. Amino acid sequences of RNases Bi and Bth are identical except for the substitution of Ala¹⁰⁶ in RNase Bth for Thr¹⁰⁶ in RNase Bi. The structural similarity of RNases Bi and Ba is about 83%.

Recently, we have isolated a strain producing high levels of extracellular RNase, which was identified according to its morphological and biochemical properties as *B. circulans*, and the procedure of purification of the protein (RNase Bci) has been developed [6]. In this paper, we report the results of determination of the complete primary structure of RNase Bci and some catalytic properties of the enzyme.

2. MATERIALS AND METHODS

Extracellular RNase Bci was isolated from the filtrate of cultural medium of *B. circulans*, strain BCF 247 [6]. RNase Ba was a product of gene-engineering technology [7]. *Staphylococcus aureus* protease V8, polynucleotides, GpC and cyanogen bromide were purchased from Sigma, and the sequence grade chemicals were from Applied Biosystems.

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Abbreviations: RNase Bci, Ba, Bi and Bth, ribonucleases of *Bacillus circulans*, *B. amyloliquefaciens*, *B. intermedius* and *B. thuringiensis* (EC 3.1.27.1), respectively.

Proteolysis of the RNases with *S. aureus* protease was performed in 10 mM potassium-phosphate buffer, pH 7.5, at 37°C for 7 h and an enzyme-to-substrate ratio of 1:50. Subdigestion with cyanogen bromide was made in 1:1 mixture of heptafluorobutyric and 88% formic acid at 25°C in the dark for 24 h and at a 1:1,000 molar peptide/reagent ratio.

Automatic Edman degradation were carried out on a Knauer Model 816 Protein Sequencer, equipped with an on-line Model 120 PTH Applied Biosystem Analyzer.

Molecular mass analyses of RNase Bci and peptides were performed on the time-of-flight spectrometers using methods of electrospray mass spectrometry [8] and plasma-desorption mass spectrometry with a Californium-252 source [9].

3. RESULTS AND DISCUSSION

Determination of the amino acid sequence of RNase Bci was based on the assumption of the high level of similarity between the extracellular *Bacillus* RNases. Only one digestion of the RNase Bci with Glu-specific *S. aureus* protease was used to elucidate the protein primary structure.

According to electrospray mass spectrometry data the relative molecular mass of RNase Bci (M_r 12,384 ± 5) closes to RNase Ba mass (M_r 12,383) calculated from its amino acid sequence [2]. RNases Bci and Ba have the same amino acid sequence for the N-terminal 25 amino acid-long regions with the exception of one substitution of Gln¹⁵ in RNase Ba for Leu¹⁵ in RNase Bci [6].

Individual peptides of RNase Bci digestion with *S. aureus* protease (SP-peptides) were isolated using the reversed-phase HPLC. Chromatographic elution patterns of RNases Bci and Ba hydrolysates coincide qualitatively (Fig. 1), and only the positions on chromatograms of the peptides SP-4 differ radically. There are four major components in the fractionating peptide mixtures and this number is in agreement with the spec-

Table II

Kinetic parameters of the transesterification reactions ($I = 0.2$ M, pH 6.2, 25°C)

RNase	Substrate	k_{cat} (s ⁻¹)	$K_m \cdot 10^4$ (M)	k_{cat}/K_m (M ⁻¹ · s ⁻¹)
Bci	poly(I)	870	0.9	$1.0 \cdot 10^7$
	poly(A)	7.8	1.6	$4.9 \cdot 10^4$
	GpC	0.1	5.3	190
Ba	poly(I)	1,200	1.1	$1.1 \cdot 10^7$
	poly(A)	16	4.4	$3.6 \cdot 10^4$
	GpC	0.5	33	160

not cleave cyclophosphates of adenosine, uridine and cytidine. The RNases lose its guanyl specificity when homopolynucleotides are used as substrates and cleave not only poly(I), but also poly(A) and poly(U), although poly(I) is the best substrate (Table II). Thus, in this case the RNases are rather guanyl-preferential.

The similarity of extracellular *Bacillus* RNases strongly argues that the enzymes are derived from a common ancestor.

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