The complete amino acid sequence of ribonuclease C_2 from *Aspergillus clavatus*

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The complete amino acid sequence of ribonuclease C_2 from *Aspergillus clavatus* has been determined. It consists of 104 amino acids, corresponding to M_r 11242. The sequence is homologous to those of fungal ribonucleases with known primary structures.

Ribonuclease C_2 Aspergillus clavatus *Amino acid sequence*

1. INTRODUCTION

Ribonuclease C_2 [RNase C_2, EC 3.1.27.3] from *Aspergillus clavatus* deprades single-stranded RNA to yield oligonucleotides with terminal guanosine 3'-phosphate [1]. As RNase T_1 *Aspergillus oryzae* RNase C_2 is a tool in nucleic acid chemistry and a model compound in the study of the recognition of nucleic acids by proteins. The crystallographic investigations of the RNase C_2 and the RNase C_2-2'(3')-guanylic acid complex are in progress [2,3].

This study has determined the primary structure of the extracellular RNase C_2 from *Aspergillus clavatus*.

2. MATERIALS AND METHODS

RNase C_2 was purified to homogeneity essentially as in [4]. After reduction and *S*-alkylation as in [5] the protein was digested with trypsin (Serva) in 0.05 M triethylamine-bicarbonate (TEAB) (pH 8.05) at 37°C for 4 h with an enzyme: protein ratio 1: 25 (w/w).

The peptides were fractionated by ion-exchange chromatography on Chromo-beads (Technicon, Ireland) cation exchanger, QAE-Sephadex A-50 and thin-layer chromatography on Cellulose TLC (Serva).

The amino acid composition of the protein and peptides was determined using Durrum D-500 analyser (USA) after hydrolysis of samples with 5.7 M HCl for 24 h at 105°C.

The amino acid sequence of the N-terminal part of the protein was established on a Beckman 890 C automatic sequenator [6]. The sequences of the large peptides were determined with polybrene [7].

PTH derivatives of the amino acids were identified by thin-layer chromatography on Kiselgel 60 F_{254} (Merc 5554) [8] with subsequent scanning on an Opton spectrophotometer and by gas–liquid chromatography [9].

The experimental details of peptide separation and the sequence determination will be published elsewhere.

3. RESULTS AND DISCUSSION

RNase C_2 from *Asp. clavatus* is a small acidic protein with an $M_r \sim 11\,000$. It contains 104 amino acid residues, including 4 arginines.

The denatured protein was submitted to automatic Edman degradation [6], and 45 out of the first 50 residues were unequivocally identified. The remaining 5 residues were identified upon analysis of the small tryptic peptides.

Arrangement of all amino acid residues within the polypeptide chain was based largely on the

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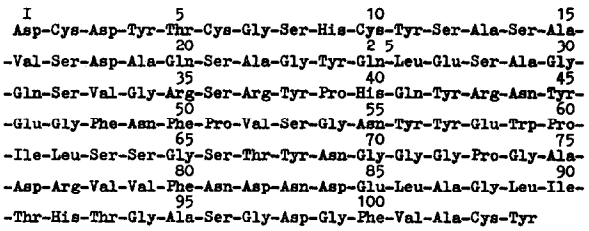

Fig. 1. RNase C_2 amino acid sequence.

automated Edman degradation of the *S*-carboxymethylated protein and two purified tryptic fragments (Asn_{44}–Arg_{77} and Val_{78}–Tyr_{104}) obtained from it.

The complete amino acid sequence of 104 residues of the RNase C_2 is shown in fig. 1.

The amino acid composition calculated from the RNase C_2 sequence is:
$Asp_7Asn_6Thr_4Ser_{13}Glu_4Gln_4Pro_4Gly_{14}Ala_9Cys_4$-
$Val_6Ile_2Leu_4Tyr_{10}Phe_4His_3Arg_4Trp_1$,
that corresponds to M_r 11 241.67.

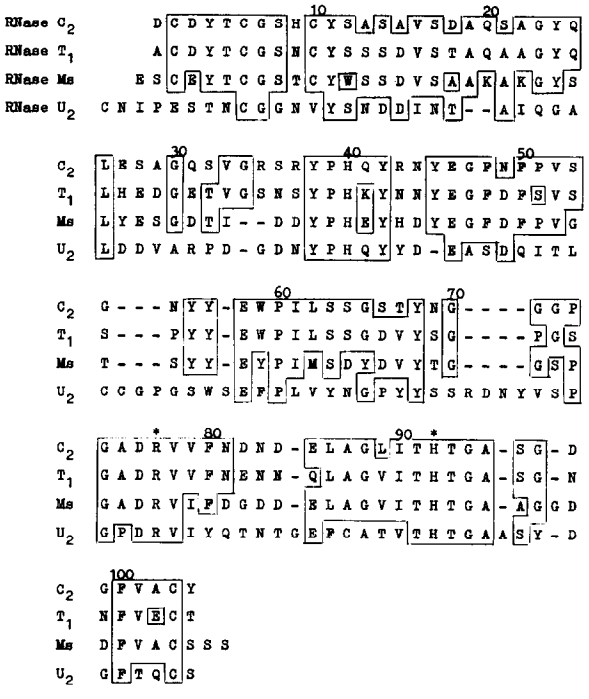

Fig. 2. Sequence comparison of RNase C_2 with RNase T_1 [10], RNase Ms [11], and RNase U_2 [12]. Residues are indicated by the single-letter code as recommended in [13]. Homologous parts of sequences are boxed. A few gaps have been placed in the sequences to achieve maximum identity; (*) catalytic residues.

Sequence comparison of the RNase C_2 with RNases of known primary structure from other fungi, namely RNase T_1 from *Asp. oryzae* [10], RNase Ms from *Aspergillus saitoi* [11], and RNase U_2 from *Ustilago sphaerogena* [12] is shown in fig. 2.

All cysteine residues (2,6,10,103) and phenylalanines (48,50,80,100), almost all tyrosines (4,11, 24,38,42,45,56,57,68) majority glycines (7,23,30, 47,70,74,88,94,97) and valines (16,33,52,78,79, 101) are invariant in 3 *Aspergillus* ribonucleases. The general homology of these RNases is near 70% while RNase U_2 from *Ustilago* has only about 30% of the same amino acid residues. The homology is much stronger around the residues that form the active site (Glu 58, Arg 77, His 40 and His 92).

In spite of great phylogenetic distance between prokaryotic and eukaryotic ribonucleases they apparently belong to the same family of proteins [14,15].

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REFERENCES

[1] Bezborodova, S.I. and Bezborodov, A.M. (1979) in: Biosintes mikroorganizmamy nukleaz i proteaz, pp. 92–130 Nauka, Moskwa.
[2] Polyakov, K.M., Tischenko, G.N., Bezborodova, S.I. and Vainshtein, B.K. (1982) Dokl. AN SSSR 262, 342–346.
[3] Polyakov, K.M., Vagin, A.A., Tischenko, G.N., Bezborodova, S.I. and Vainshtein, B.K. (1983) Dokl. AN SSSR in press.
[4] Bezborodova, S.I., Beletskaya, O.P. and Grishchenko, V.M. (1977) Biokhimiya 42, 1556–1566.
[5] Crestfield, M., Moore, S. and Stein, W.H. (1963) J. Biol. Chem. 238, 622–627.
[6] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–91.
[7] Tarr, G.E., Beecher, J.F., Bell, M. and McKean, D.J. (1978) Anal. Biochem. 84, 622–627.
[8] Alakhov, Yu.B., Vinokurov, L.M., Dovgas, N.V. and Motuz, L.P. (1983) Bioorg. Khim. 9, 293–303.
[9] Pisano, J.J. and Bronzert, T.J. (1969) J. Biol. Chem. 244, 5597–5607.

[10] Takahashi, K. (1971) J. Biochem. 70, 617–634.
[11] Watanabe, H., Ohgi, K. and Irie, M. (1982) J. Biochem. 91, 1495–1509.
[12] Sato, S. and Uchida, T. (1975) Biochem. J. 145, 353–360.
[13] IUPAC/IUB Recommendations (1968) Eur. J. Biochem. 5, 151–153.
[14] Hartley, R.W. (1980) J. Mol. Evol. 15, 355–358.
[15] Mauguen, Y., Hartley, R.W., Dodson, E.J., Dodson, G.G., Bricogne, G., Chotia, C. and Jack, A. (1982) Nature 297, 162–164.