RIBONUCLEASE BS-1: SEQUENCE OF TWO CYANOGEN BROMIDE PEPTIDES

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SUMMARY

When native RNAase BS-1[x] is treated with cyanogen bromide, one peptide (CB2) is cleaved from the rest of the protein (CB1) and can be isolated by gel filtration. Peptide CB2 has been sequenced and shown to be the N-terminal peptide of the protein, strictly homologous to the peptide 1-13 of RNAase A. A second fragment (peptide PF2) can be separated by gel filtration after performic acid oxidation of the CB1 fragment. The sequence of this peptide is partially homologous to the 14-29 sequence of RNAase A. From the recovery values determined for peptides CB2 and PF2, it appears that each peptide is present in both chains of RNAase BS-1. These and previous findings are briefly discussed in terms of structure to function relationships.

Ribonuclease BS-1 has been shown by work from this Laboratory to be very similar to pancreatic RNAase A in its main enzymatic properties, like substrate specificity, pH optimum, apparent K_m, etc., while the molecular weight is about twice and the amino acid composition shows significant differences[2,3]. Furthermore, it has also been found that the enzyme is made up of two subunits which are probably identical, both with lysine as the N-terminal residue and with a sequence of VAL-HIS-PHE-ASP-ALA-SER-VAL at the C-terminal end. The end groups and the C-terminal sequence of both chains are identical with those of RNAase A, but RNAase BS-1 may not be considered a dimer of RNAase A[4].

MATERIALS AND METHODS

RNAase BS-1 was purified as previously described[2]. Trypsin-TPCK, DFP-carboxypeptidase A and α-Chymotrypsin were products of Worthington Biochemical Corporation.

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[x] RNAase BS-1 is the major component of bull semen ribonuclease activity[1]; a second component called RNAase BS-2 has also been found[2]. Other non-standard abbreviations: RNAase A, bovine pancreatic ribonuclease (E.C. 2.7.7.16); TPCK, L-(1-tosylamido-2-phenyl) ethyl chloromethyl ketone; DFP, diisopropyl phosphofluoridate; HSer, homoserine; CysA, cysteic acid; PTH, 3-phenyl-2-thiohydantoin; dansyl, 1-dimethylaminonaphtalene-5-sulfonyl.

Various conditions were tested for the reaction of cyanogen bromide with RNAase BS-1[5,6]. Cleavage was most extensive when a 100-fold molar excess (with respect to methionine) of cyanogen bromide was added to RNAase BS-1 (50 to 150 mg) dissolved in 5 ml of 70% formic acid, and reaction was allowed to proceed for 30 hours at 30°. After dilution and repeated lyophylization, the extent of cleavage was measured by amino acid analysis.

Amino acid sequences were determined by subtractive Edman degradation, as outlined by Konigsberg[7], and by the dansyl-Edman procedure of Hartley[8].

High voltage electrophoresis was carried out in a Gilson apparatus with pyridine-acetate buffers at pH 6.5[9] and pH 3.6[10], using the cadmium-ninhydrin reagent of Barrolier[11] for the detection of peptides.

Performic acid oxidation and amino acid analyses were performed by standard methods, as previously described[2].

Amide group assignment was based (1) on peptide electrophoretic mobility[12], (2) on the amino acid analysis of carboxypeptidase A digests and (3) on the analysis of PTH-derivatives of N-terminal amino acids, after Edman degradation.

The nomenclature of peptides was based on the method used for isolation; in this paper CB, CH, T and PF denote peptides obtained after cyanogen bromide cleavage, chymotryptic or tryptic digestion, and performic acid oxidation, respectively.

RESULTS AND DISCUSSION

When native RNAase BS-1 is treated with cyanogen bromide, 85 to 95 per cent of the protein methionyl residues are reacted, as judged by amino acid analysis. Fractionation of the reaction product was carried out by gel filtration on a Sephadex G-25 column in 0.1 N acetic acid (Fig. 1). The elution profile showed three peaks which were analyzed by paper electrophoresis and amino acid analysis. The first peak (CB1), which consisted of the major part of cleaved protein in which the disulfide bridges still hold together the resulting peptides, and also of unreacted protein, was set aside. The second peak was found to be a peptide (peptide CB2), while the third fraction consisted of free homoserine[x].

[x]Unless otherwise stated, in this report "homoserine", whether free amino acid or C-terminal residue of a peptide, stands for the mixture of homoserine and homoserine lactone.

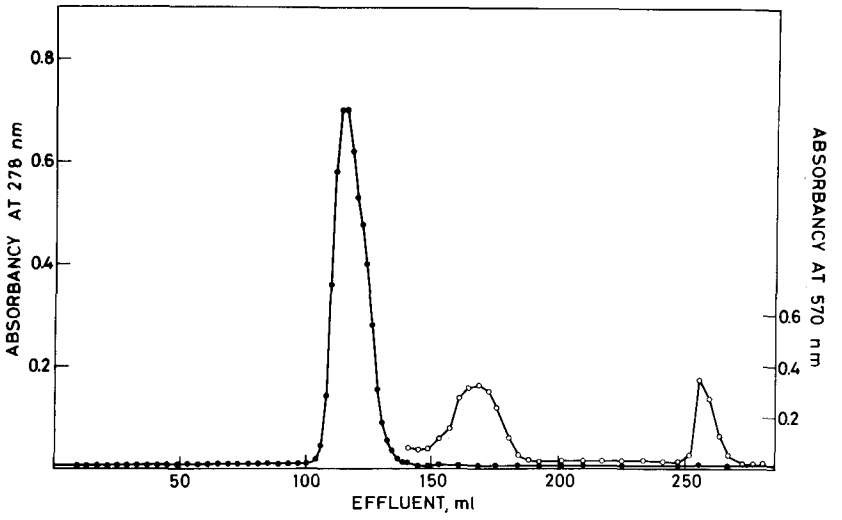


Fig. 1 - Gel filtration of CNBr peptides on a 1.5 x 140 cm column of Sephadex G-25. •-•-•, absorbancy at 278 nm; o-o-o, absorbancy at 570 nm after ninhydrin reaction of 0.1 ml fraction aliquots. Peptide CB1 is the peak eluted at ml 150; the homoserine fraction is the peak eluted at ml 250.

TABLE 1

Amino acid analyses of isolated peptides

	CB2	CB2-T1	CB2-T2	CB2-T3	CB1-PF2	CB1-PF2-CH1	CB1-PF2-CH2
Lysine	2.00	2.00					
Histidine	0.95			1.11			
Arginine	1.04		1.00				
Cysteic Acid					0.92		0.75
Aspartic Acid					4.00	2.62	1.00
Serine	1.10	0.93			5.95	5.54	
Homoserine	0.87			1.00	0.70		0.85
Glutamic Acid	3.06	1.03	1.08	1.11			
Proline					0.95	1.07	
Glycine					1.05	1.00	
Alanine	2.91	3.10					
Leucine					1.00		0.94
Tyrosine					0.92	0.78	
Phenylalanine	0.89		0.95				

The results are presented as molar ratios.

Peptide CB2 was found to be pure by paper electrophoresis at pH 6.5 and pH 3.6, carried out immediately after treatment with trifluoroacetic acid[13] in order to convert any C-terminal homoserine residues to the lactone form. Its amino acid composition is reported in Table 1. Reaction of the peptide with dansyl chloride showed lysine as the N-terminal residue.

CB2 was then subjected to trypsin hydrolysis (10µg trypsin per mg peptide) for 6 hours at 22° in 0.1 M ammonium bicarbonate, and the hydrolysate fractionated either by paper electrophoresis at pH 6.5 or on a column of Dowex 50-X2, eluting with an acetate-pyridine gradient system[14]. Three peptide fragments were obtained: CB2-T1, CB2-T2 and CB2-T3. By subtractive Edman degradation (see Table 2), and after assignment of the amide groups, the following sequences resulted:

Peptide CB2-T1 LYS-GLU-SER-ALA-ALA-ALA-LYS

Peptide CB2-T2 PHE-GLU-ARG

Peptide CB2-T3 GLN-HIS-MET

TABLE 2

Subtractive Edman Degradation

		Amino Acid Composition							
		Lys	His	Arg	Ser	HSer	Glx	Ala	Phe
CB2-T1		2.00			0.93		1.03	3.10	
	Step 1	1.00			1.04		0.93	3.12	
	2	1.00			0.96		0.08	3.20	
	3	1.00			0.06		0.05	3.08	
CB2-T2				1.00			1.08		0.95
	Step 1			1.00			0.92		0.06
	2			1.00			0.08		0.05
CB2-T3			1.11			1.00	1.11		
	Step 1		1.28			1.00	0.05		
	2		0.05			1.00	0.02		

The results are presented as molar ratios. The amino acid residue removed at each step is underlined.

As it appears from Table 2, the sequence of the last four amino acid residues of peptide CB2-T1 has not been determined by Edman degradation. Lysine being the C-terminal residue, the only possible sequence of these residues is as shown above.

Since lysine had already been found to be the N-terminal residue of the intact CB2 peptide, and since methionine, converted to homoserine by the cyanogen bromide reaction, must be the C-terminal residue, the complete sequence of peptide CB2 is:

LYS-GLU-SER-ALA-ALA-ALA-LYS-PHE-GLU-ARG-GLN-HIS-MET

From this sequence, the close similarity to the 1-13 sequence in RNAase A is evident; in fact, the only difference is represented by SER-3, which substitutes for THR-3. Therefore, given the reported finding of lysine as the N-terminal residue in RNAase BS-1[4], the sequence just described strongly suggests that peptide CB2 is the N-terminal peptide. This conclusion is particularly strengthened by the comparison with the cyanogen bromide cleavage of RNAase A, which also gives, as main product of the reaction on the native protein, the N-terminal peptide[5].

As a next step, fraction CB1 was oxidized with performic acid. The product of the reaction was taken to a column of Sephadex G-25 and eluted by 0.1 N acetic acid. The elution profile showed one large fraction composed of unresolved components (CB1-PF1), and a minor one (CB1-PF2). Attention was focused on this second fraction which contained a single peptide, as judged by paper electrophoresis at pH 6.5 and pH 3.5,

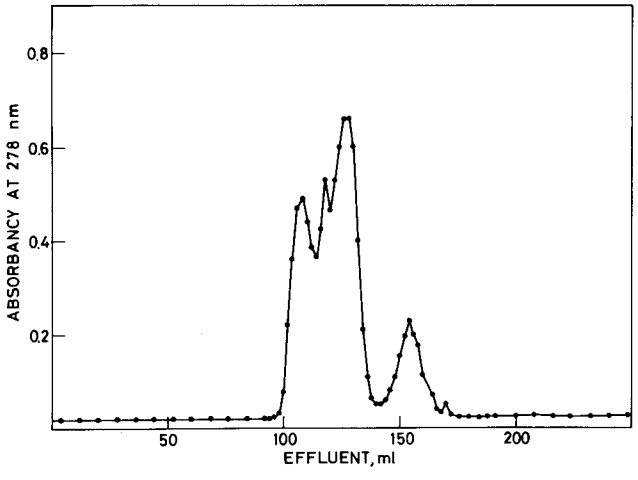
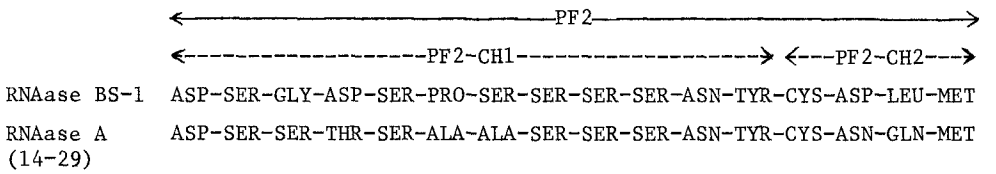


Fig. 2 - Gel filtration of performic acid oxidized CB1 fraction on a 1.5 x 140 cm column of Sephadex G-25. Peptide PF2 is the peak eluted at ml 140.

with aspartic acid as N-terminal residue. Its amino acid composition is reported in Table 1. The sequence of peptide CB1-PF2 was determined by the dansyl-Edman procedure, carried out on the intact peptide and on two fragments (CB1-PF2-CH1 and CB1-PF2-CH2), obtained by digestion with chymotrypsin of PF2 (3 hours at 37° in 0.1 M ammonium bicarbonate at an enzyme/peptide ratio of 1/50, w/w). The amino acid composition of these peptides is also reported in Table 1. After assignment of amide groups, the following sequence resulted, as given below along with the 14-29 sequence of RNAase A for comparison



The degree of homology between peptide PF2 of RNAase BS-1 and the peptide 14-29 of RNAase A is not as high as for the N-terminal peptides of the two proteins, but appears high enough to suggest that peptide CB2 and peptide PF2 are contiguous.

Given the amino acid sequences reported above, it seemed interesting to calculate the yields of the two peptides from the original protein. It was found for peptide CB2 a yield of 1.7 moles per mole of protein and for peptide PF2 of 1.2 moles per mole of protein. From these values it would appear that the two sequences are each present in both chains of RNAase BS-1, a conclusion which supports the identity between the two chains.

Furthermore, as a histidine residue has already been found in RNAase BS-1 in a sequence position equivalent to that of HIS-119 of RNAase A[4], the finding reported here of a HIS-12 in RNAase BS-1 would support the conclusion that both RNAases, pancreatic and seminal, have identical or very similar active sites. The hypothesis is based on the location of both HIS-12 and HIS-119 at the active site of RNAase A (see Ref. 15 for a review) and is in line with the strong similarity between the enzymatic actions of the two enzymes on single-stranded RNA[2]. The number and the availability of active sites in RNAase BS-1, and the structural basis for its action on double-stranded RNA[16] may only come from further studies now in progress in this Laboratory.

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