THE AMINO ACID SEQUENCE OF GNU PANCREATIC RIBONUCLEASE

Gerda GROEN, Gjalt W. WELLING and Jaap J. BEINTEMA
Biochemisch Laboratorium, Rijksuniversiteit, Zernikelaan, de Paddepoel, Groningen, The Netherlands

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1. Introduction

During the course of a study on the evolutionary history of pancreatic ribonucleases, several species belonging to the family of the *Bovidae* have been investigated. The amino acid sequences of four pancreatic ribonucleases from two subfamilies: the *Bovinae*, (cow [1] and American bison (F. J. Muskiet, G. W. Welling and J. J. Beintema, unpublished) and [2]) and the *Caprinae* (sheep [3,4] and goat [4]) have been determined.

Here we present primary structure of pancreatic ribonuclease from brindled gnu or wildebeest (*Connochaetes taurinus*), member of another subfamily of the *Bovidae*, the *Hippotraginae.*

2. Material and methods

Material and methods were as described by Welling et al. [5] unless otherwise stated.

Gnu ribonuclease was isolated by affinity chromatography. After digestion with different proteolytic enzymes, peptides were isolated by gel filtration on Sephadex G-25 (fine grade) or Sephadex G-50 (fine grade), preparative paper electrophoresis at pH 3.5 and paper chromatography (BAWP). Peptides were sequenced by the method of Gray for extended degradations [6]. Isolated peptides were numbered according to their position in the polypeptide chain. Gnu ribonuclease (10 mg) was cleaved with trypsin after reduction and aminoethylation. Peptides from this digest were prefixed by the letter A. Gnu ribonuclease (30 mg) was oxidized with performic acid and digested with trypsin in 0.2 M ammonium acetate, pH 8.0 (E : S = 1 : 50, by weight) for 2 h. Peptides resulting from this digest were prefixed by the letter T. The oxidised protein (20 mg) was also digested with thermolysin. Peptides from this digest were prefixed by the letter H. Native gnu ribonuclease (4 mg) was cleaved with subtilopeptidase A. The fragment obtained from this cleavage was denoted by the letter S. Native gnu ribonuclease (15 mg) was also treated with cyanogen bromide. The peptides obtained by gel filtration before and after performic acid oxidation are prefixed by the letter B. Secondary cleavage of the remaining large fragments was performed with 100 μl of chymotrypsin (1 mg/ml) in 0.2 M *N*-ethylmorpholine-acetate, pH 8.0, at 37°C for 2 h.

An impure tryptic peptide (residues 105–124) was digested with 10 μl of chymotrypsin (1 mg/ml) in 0.2 M *N*-ethylmorpholine-acetate, pH 8.0, at 37°C for 2 h. The peptides from these digests were prefixed by the letter C.

3. Results and discussion

Determination of the N-terminal residue of gnu ribonuclease yielded only lysine. Table 1 gives the amino acid compositions of the tryptic peptides. Table 2 gives the amino acid compositions of the peptides obtained after cleavage with thermolysin, subtilisin, cyanogen bromide and of the peptides from the secondary cleavage with chymotrypsin.

The amino acid compositions of the isolated peptides, pointed to a close relationship between gnu and bovine ribonuclease. For this reason it was decided not to isolate all overlapping peptides, but to infer the location of the fragments by homology with the completely sequenced bovine ribonuclease [1]. Peptides from gnu ribonuclease differing in amino acid

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Table 1
Amino acid compositions of tryptic peptides (T and A) of gnu ribonuclease

Peptide	T1[a]	T2	T3[a]	T4[a]	T5[a]	T6	T7[a]	T8	T9	T10[a]	A1	A2
Cysteic acid	[b] (1)			[b] (1)	[b] (2)	[b] (1)	[b] (1)		[b] (1)			
Asp	2.8 (3)		2.0 (2)	1.0 (1)	2.5 (2)	1.8 (2)	1.0 (1)		0.8 (1)		2.1 (2)	
Met $(O)_2$	1.7 (3)						0.7 (1)			0.5		
Thr	1.9 (1)		0.9 (1)	0.9 (1)	0.6 (1)	1.0 (1)	1.5 (2)	0.7 (1)		0.6 (1)	1.0 (1)	
Ser	5.4 (7)	0.9 (1)	1.5		1.3 (1)	1.0 (1)	1.0 (2)	2.1 (2)		0.4	0.7[c]	
Glu	2.6 (2)		3.0 (1)	0.8	3.7 (3)	1.9 (2)	0.5	1.0 (1)		1.2 (1)	2.0 (2)	
Pro				1.3 (1)	1.3 (2)		0.4		1.2 (1)		2.2 (2)	
Gly			0.9			1.1 (1)		2.0[c] (1)		0.5		
Ala	1.9 (1)		1.9	0.6	3.2 (2)		0.6		1.0 (1)	1.6 (2)	1.9 (2)	
Val				1.0 (1)	2.8 (4)						3.8 (4)	
Ile							0.8 (1)			0.4		
Leu			2.1 (1)		1.0 (1)					0.4	1.0 (1)	
Tyr	[b] (1)					1.6 (2)			[b] (2)			
Phe				0.7 (1)	0.5 (1)						1.0 (1)	
Lys	1.0 (1)		0.8	1.3 (1)	1.7 (2)		0.5	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	[b] (1)
AetCys											n.d.(2)	
His	1.0 (1)				1.1 (1)						1.0 (1)	
Arg		1.1 (1)	0.8 (1)				0.9 (1)					
Position in seq.	11–31	32–33	34–39	40–46	40–61	67–76	77–85	86–91	92–98	99–103	40–58	66 and 104

The compositions of peptides derived from the amino acid sequence are shown in parentheses.

[a] Relatively impure peptide. Peptide T1 and T3 were only necessary to get evidence for lysine 31 and asparagine 34, respectively. T3 is probably a mixture of 34–39 and 2–7. The dansyl-Edman results of peptides T4, T7 (contaminated with T9) and T10 were satisfactory.

[b] Not determined quantitatively.

[c] Peptides eluted from paper were sometimes contaminated with glycine and serine.

n.d., not determined

Table 2
Amino acid compositions of thermolysin peptides (H), subtilisin peptide (S), cyanogen bromide peptides (B) and chymotryptic peptides (C)

Peptide	H1	H2	H3	H4	S	B1	B2[a]	C1[a]	C2[a]	C3	C4
Cysteic acid	[b] (1)	[b] (2)		[b] (1)			n.d. (1)		[b] (3)		
Asp	1.0 (1)	2.1 (2)		1.0 (1)	1.5 (1)		2.6 (3)		3.8 (4)		1.1 (1)
Thr	1.0 (1)	1.4 (2)	1.0 (1)		1.0 (1)		1.0 (1)	0.6	1.2 (1)		
Ser	0.4	2.2 (2)		0.3	5.0 (5)	0.9 (1)	6.6 (7)		2.8 (1)		1.0 (1)
Glu	1.1 (1)	1.4 (1)	1.2 (1)	1.2 (1)	3.1 (3)	2.5 (3)	2.2 (1)	1.0 (1)	1.6 (3)		
Hse (lactone)						[c] (1)	[c] (1)				
Pro	1.0 (1)	0.6 (1)		1.3 (1)				1.0 (1)		1.1 (1)	
Gly		0.6 (1)		1.0 (1)	0.3			0.4	1.2 (1)		
Ala		0.9 (1)	1.7 (2)	1.0 (1)	4.0 (3)	2.6 (3)	1.3 (1)		3.1 (3)		1.1 (1)
Val				1.0 (1)				0.6 (1)	2.5 (3)	1.9 (2)	0.7 (1)
Met					0.7 (1)						
Ile		0.5 (1)		0.3[d] (2)							
Leu	0.7 (1)							1.0 (1)			
Tyr		[b] (2)		[b] (1)			1.6 (1)		0.9 (1)		
Phe					1.0 (1)	1.2 (1)				0.9 (1)	
Lys	1.0 (1)	1.6 (2)	2.2 (2)		1.8 (2)	1.9 (2)	0.4	1.1	2.0 (2)		
His			0.9 (1)		0.9 (1)	1.1 (1)		1.0 (1)		1.0 (1)	
Arg	1.0 (1)	1.0 (1)			1.2 (1)	1.0 (1)	0.3				
Position in seq.	35–42	81–98	99–105	106–115	1–20	1–13	14–29	47–51	52–73	116–120	121–124

The compositions of peptides derived from the amino acid sequence are shown in parentheses.

[a] Relatively impure peptide. The dansyl-Edman results of B2 and C1 were satisfactory.

[b] Not determined quantitatively.

[c] Homoserine elutes at the position of glutamic acid (peptide B2). In peptide B1 only homoserinelactone was observed.

[d] Ile–Ile bonds are not hydrolysed completely in 20 h.

n.d., not determined.

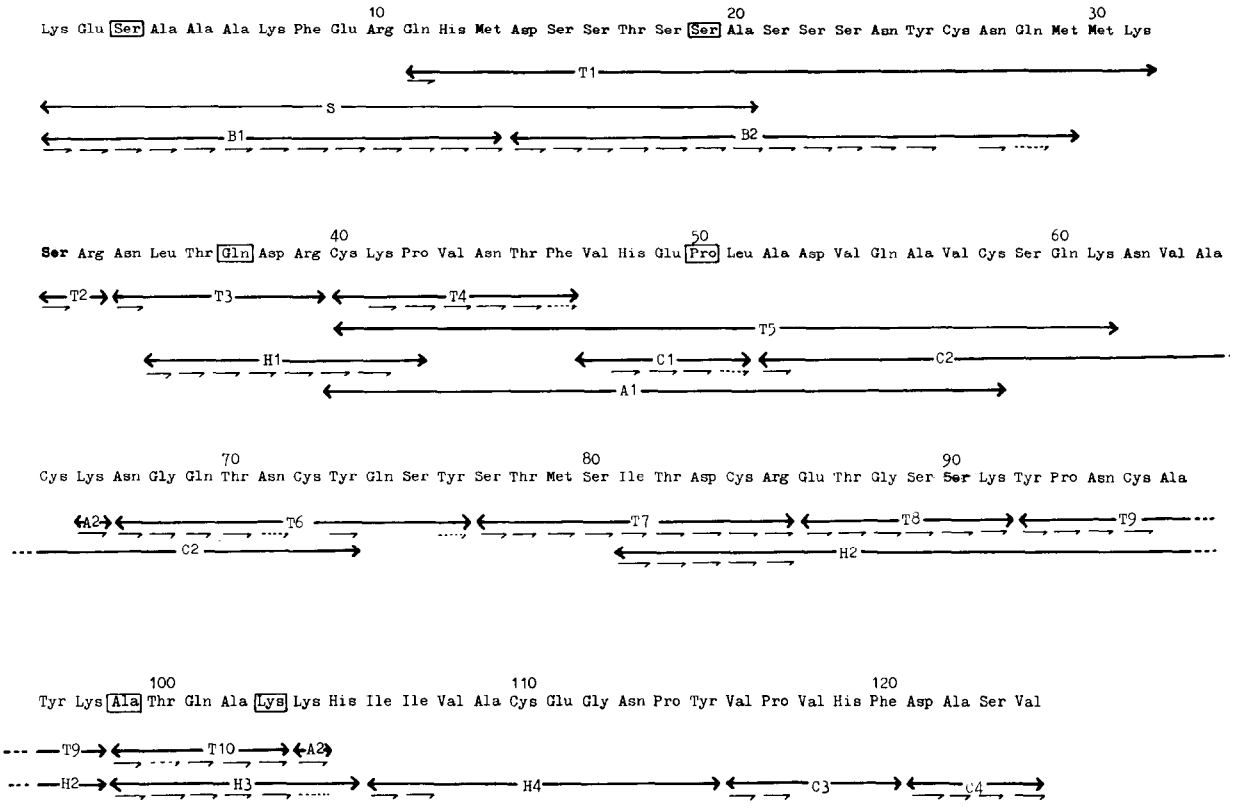

Fig.1. Amino acid sequence of gnu pancreatic ribonuclease. The sequence was partly determined by the dansyl-Edman technique (——). Dashed lines indicate results which were not quite certain. Peptides were positioned by homology with bovine ribonuclease. Those amino acids differing from bovine ribonuclease are indicated in blocks. For peptide nomenclature, see under Materials and methods.

composition from homologous peptides in one of the four *Bovidae* ribonucleases were sequenced next to some other peptides. The alignment and the sequence results are shown in fig.1. Amide positions were not determined and maximum homology was assumed with the other *Bovidae* ribonucleases.

We got satisfactory evidence for the complete sequence, with exception of the residues 59–65, which were part of two large peptides T5 and C2 (see tables 1 and 2). These two peptides were not obtained in pure form, but the analytical values are complementary to each other. From these data it can be concluded that gnu ribonuclease does not differ from other *Bovidae* ribonucleases in the sequence 59–65.

Six differences in amino acid sequence were found compared with bovine and bison ribonuclease and three when compared with sheep and goat ribonuclease (see table 3). In bovine ribonuclease, these positions are located at the surface of the molecule. Two of the replacements are rather unusual. Proline is an uncommon amino acid at position 50 in the

Table 3
Differences between goat/sheep, bison/cow and gnu ribonuclease

Position	3	19	37	50	99	103
Goat/sheep RNase	Ser	Ser	Gln	Ser	Thr	Glu
Cow/bison RNase	Thr	Ala	Lys	Ser	Thr	Asn
Gnu RNase	Ser	Ser	Gln	Pro	Ala	Lys

Table 4
Difference matrix of ribonucleases

	Gnu	Cow	Sheep	Giraffe	Reindeer
Gnu	–	6	3	10	13
Cow/bison	5%	–	4	11	14
Sheep/goat	2%	3%	–	7	10
Giraffe	8%	9%	6%	–	14
Reindeer	10%	11%	8%	11%	–

Calculated from amino acid sequences determined for the enzymes from cow [1], bison ([2], F. J. Muskiet, G. W. Welling and J. J. Beintema, unpublished), sheep [3], goat [4], giraffe [11], reindeer [10] and gnu (this article). Both the number and percentage of differences are shown.

artiodactyl ribonucleases and is only found at that position in horse ribonuclease [7] and in four out of five rodent ribonucleases [8,9]. A lysine at position 103 is only found at that position in dromedary ribonuclease [5] and in topi (*Damaliscus korrigum*) ribonuclease (H. Kuper and J. J. Beintema, unpublished). The topi is a very close relative of the gnu and belongs to the same tribe, the *Alcelaphini*.

A difference matrix of ribonucleases from the *Cervidae* [10], the giraffe [11] and the *Bovidae*, is shown in table 4. Obviously these ribonucleases roughly group together in families, subfamilies and tribes according to their zoological classification [12,13] (See table 5). A larger number of differences is found between ribonucleases from species of different families than between those belonging to the same subfamily.

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Table 5
Zoological classification of *Cervidae, Bovidae, Giraffidae* mentioned in this study, according to Morris [13]

Family	Subfamily	Tribe	Species
Bovidae	*Caprinae*	*Caprini*	Sheep/goat
	Bovinae	*Bovini*	Cow/bison
	Hippotraginae	*Hippotragini*	Gnu/topi
Giraffidae	*Giraffinae*		Giraffe
Cervidae	*Odocoileinae*	*Rangiferini*	Reindeer

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