THE AMINO ACID SEQUENCES OF REINDEER, MOOSE AND FALLOW DEER PANCREATIC RIBONUCLEASES

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1. Introduction

Like other true ruminants, deer have a fairly high ribonuclease content in their pancreas [1,2] and are very suitable to be included in evolutionary studies of pancreatic ribonucleases. Zwiers et al. have determined the primary structures of red deer and roe deer ribonuclease [3]. In this article we present the determination of the primary structures of the enzymes from three other deer species: reindeer (*Rangifer tarandus*), moose or European elk (*Alces alces*) and fallow deer (*Dama dama*). The results of these studies necessitated a reinvestigation of a part of the primary structures of red deer and roe deer ribonuclease to correct the sequences of the residues 15–23 of these enzymes [4,5]. Moose ribonuclease is a glycoprotein; reindeer and fallow deer ribonuclease exist only in a carbohydrate-free form.

2. Materials and methods

All methods and materials were the same as described by Gaastra et al. [6].

3. Results and discussion

Isolation of reindeer ribonuclease by ion exchange chromatography on CM-cellulose [7,8] was unsuccessful, probably because of the small excess of positive charges in this enzyme, compared to most other ribonucleases. However, the three ribonucleases could be purified by affinity chromatography [9]. 300 mg reindeer ribonuclease were obtained from 850 g pancreas (25 animals), 15 mg moose ribonuclease from a single pancreas (135 g) and 30 mg fallow deer ribonuclease from 165 g pancreas (7 animals).

Determination of the N-terminal residues by dansylation yielded only lysine. Reindeer and fallow deer ribonuclease are not glycosidated and showed one single band upon polyacrylamide gel electrophoresis. Moose ribonuclease contained carbohydrate (about 6 mannose equivalents, as tested by the orcinol reaction [10]) and showed three bands upon polyacrylamide gel electrophoresis, all enzymatically active [11]. Chromatography on CM-cellulose [3,8] of purified moose ribonuclease showed that no carbohydrate-free components were present.

100 mg reindeer ribonuclease, 5 mg moose ribonuclease and 30 mg fallow deer ribonuclease were used for the determination of the primary structures. The peptides obtained after tryptic cleavage of the reduced and aminoethylated proteins were purified by gel filtration on Sephadex G-25 and by ion exchange chromatography on Aminex A-5 [6]. The amino acid compositions of the peptides are shown in table 1 and the locations were inferred from homology with other ribonucleases (fig.1).

The peptides obtained from secondary cleavages with thermolysin of peptides T3 from moose and fallow deer ribonuclease and peptide T6 from reindeer ribonuclease were purified by preparative electrophoresis at pH 3.5. The amino acid compositions of these peptides are shown in table 2.

Only peptides T3, T5, T12 and T15, which differed in amino acid composition between the three species, were used for further amino acid sequence studies. The sequence of peptide T3 from reindeer ribonuclease could be determined unambiguously by dansyl-Edman

Table 1

The amino acid composition of peptides obtained after digestion of aminoethylated pancreatic deer ribonucleases by trypsin

	T1			T2			T3			T4			T5			T5b	T6		
	R	M	F	R	M	F	R	M	F	R	M	F	R	M	F	F	R	M	F
Asp							2.1(2)	2.0(2)	2.4(2)	1.2	1.2	1.2 (1)	2.0(2)	2.0(2)	1.1(1)	1.1(1)	2.2	2.4	2.0 (2)
Thr													1.0(1)	0.9(1)	0.9(1)	0.9(1)	0.9	1.0	0.9 (1)
Ser	1.0	1.2	1.0 (1)				6.2(6)	6.0(6)	5.3(6)	1.3	1.0	1.0 (1)					1.5	1.6	1.5 (1)
Glu	1.2	1.3	1.2 (1)	1.0	1.0	1.0 (1)	1.1(1)	1.1(1)	1.3(1)	1.9	1.9	2.0 (2)	1.2(1)	1.2(1)	1.1(1)	1.2(1)	2.1	2.2	2.4 (2)
Pro							2.1(2)	1.4(1)	1.1(1)		0.5						1.1	0.9	1.1 (1)
Gly						0.2													0.3
Ala	3.2	2.8	2.8 (3)				1.1(1)	1.0(1)	0.9(1)								2.1	2.1	2.2 (2)
Val																	3.6	3.6	4.0 (4)
Met							0.9(1)	1.1(1)	2.1(2)	1.6	1.8	1.4 (2)			1.0(1)	1.0(1)			
Ile								1.0(1)											
Leu													1.0(1)	0.9(1)			1.0	1.0	1.0 (1)
Tyr							0.9(1)	1.0(1)	0.9(1)										0.2
Phe				1.0	1.0	1.0 (1)											1.1	0.8	0.8 (1)
Lys	1.7	1.8	1.7 (2)							0.5	0.5	+ [a]			0.9(1)	0.3	1.0	0.8	1.0 (1)
AetCys							1.0(1)	+ (1)	+ (1)								2.0	1.3	1.9 (2)
His							1.0(1)	+ (1)	+ (1)								0.7	0.8	1.0 (1)
Arg				1.2	1.2	0.9 (1)				0.4	1.3	+ (1)	1.0(1)	0.4(1)	1.1(1)	0.9(1)			
nmoles	1470	125	520	2300	50	450	860	45	225	600	30	50	3000	110	180	250	2850	110	500
position in seq.	1-7			8-10			11-26			27-33			34-39			35-39	40-58		

	T7			T8			T9	T9	T9 T5a	T10			T11			T11b			
	R	M	F	R	M	F	R	M	F	R	M	F	R	M	F		R	M	F
Asp				1.0	1.1	1.0 (1)				1.8	1.6	1.5 (2)	2.0	2.3	2.1 (2)				
Thr													1.1	0.8	1.0 (1)				
Ser	0.4									1.0	1.3	1.0 (1)	1.8	2.0	1.9 (2)				
Glu	1.0	1.0	1.0 (1)							1.0	0.9	1.0 (1)	1.1	0.8	1.0 (1)				
Pro																			
Gly	0.5									1.1	1.2	1.1 (1)		1.1					
Ala				0.9	0.9	1.0 (1)							1.1	1.1	1.0 (1)				
Val				1.0	1.1	1.0 (1)													
Met													1.1	1.1	1.1 (1)				
Ile													1.0	1.3	1.0 (1)				
Leu																			
Tyr													1.0	1.6	1.0 (1)				
Phe	1.1	0.9	0.8 (1)																
Lys	0.9	1.1	1.0 (1)				1.0	1.0	1.0 (1)		0.3		0.3	2.3					
AetCys				0.9	1.0	0.9 (1)				0.8	1.0	0.6 (1)	1.2	0.9	1.1 (1)				
His													0.9	1.3	1.0 (1)				
Arg													1.0	+	1.2 (1)	1.0	1.0	1.0	(1)
nmoles	1800	110	650	2700	70	800	1350	200	250	2100	50	400	500	20	150	450	15	n.d.	
position in seq.	59-61			62-65			66	66	66=34	67-72			73-85			85			

	T12		T13		T12,13	T14			T15			T16			T17		
	R	F	R	F	M	R	M	F	R	M	F	R	M	F	R	M	F
Asp		1.0(1)	0.9(1)	1.0(1)	3.2(3)									0.3	2.2	2.3	1.7 (2)
Thr	0.9(1)								1.9(2)	1.7(2)	1.1(1)	0.3	0.5				
Ser	1.9(2)	1.8(2)			1.8(2)							0.4	0.3		1.3	1.0	0.8 (1)
Glu	1.0(1)	1.3(1)			1.3(1)				2.1(2)	2.1(2)	2.0(2)				1.0	1.2	1.0 (1)
Pro			1.3(1)	1.1(1)	1.0(1)										2.2	2.1	2.0 (2)
Gly	1.2(1)	1.0(1)			1.0(1)			0.2				0.4			1.1	1.1	1.1 (1)
Ala									1.2(1)	1.0(1)	1.9(2)	1.2	1.0	1.1 (1)	1.0	1.0	1.0 (1)
Val						0.9	0.9	1.0 (1)				1.1	1.0	0.9 (1)	2.7	3.3	3.0 (3)
Met																	
Ile												1.9	1.3	1.4 (2)			
Leu																	
Tyr			1.0(1)	1.1(1)	1.0(1)	0.9	1.1	1.0 (1)									0.9 1.0 1.0 (1)
Phe																	0.8 0.8 1.0 (1)
Lys	0.9(1)	1.0(1)				1.1	1.0	1.1 (1)	0.9(1)	1.1(1)	1.0(1)						
AetCys			1.0(1)	0.9(1)	0.8(1)							0.8	1.2	1.1 (1)			
His												0.9	1.2	1.0 (1)	0.9	0.8	0.9 (1)
Arg														0.3			
nmoles	2700	1200	1800	350	30	1000	60	160	2800	70	1000	3000	80	800	550	30	300
position in seq.	86-91		92-95		86-95	96-98			99-104			105-110			111-124		

The peptides were numbered according to their position in the peptide chain. (R) Reindeer; (M) Moose; (F) Fallow deer. The amino acid compositions of peptides from red deer and roe deer ribonuclease [3] are given in parentheses behind the analytical values for those peptides which are identical in the five deer ribonucleases. The amino acid compositions from sequence determinations are given in parentheses behind each analysis of the peptides which differ in the deer ribonucleases.

+ Not determined quantitatively.

a) Peptides T4 and T9 eluted close to each other from Aminex A-5. This caused the contaminations peptides T4 with lysine.

degradation of the undigested peptide and of peptide T3 from fallow deer ribonuclease by dansyl-Edman degradation of smaller peptides obtained after digestion with thermolysin. Of peptide T3 from moose ribonuclease only the amino acid compositions and the N-terminal residues of the thermolysin peptides were determined (fig.1). The other residues, including alanine 17, were positioned by homology.

Peptide T5 from moose ribonuclease is a glycopeptide. Its carbohydrate composition also was determined (table 3). Due to the small amount of material available only those residues of peptide T12, 13 from moose ribonuclease were identified which were expected to differ from reindeer ribonuclease. The C-terminal sequence of reindeer ribonuclease was determined by digestion with carboxypeptidase A (fig. 1).

Amide assignments were made by the method of Offord [12]. If a peptide contained side chains with non-amidated and with amidated carboxylic groups, the charge of the peptide was determined before and after cleavage of these residues by Edman degradation. The amide assignments in peptides T6H4, T11 and T17 in red deer and roe deer ribonuclease have not been completed in our previous study [3]. Here we have made complete amide assignments for the homologous peptides from other deer species. The results of the amide assignments are summarized in table 4. During Edman degradation lysine and aminoethylcysteine residues become neutral since the ϵ-amino group is substituted by a phenylthiocarbamyl residue. Our results are compatible with complete substitution of the side chains of these amino acid residues during Edman degradation, with the exception of peptide T12

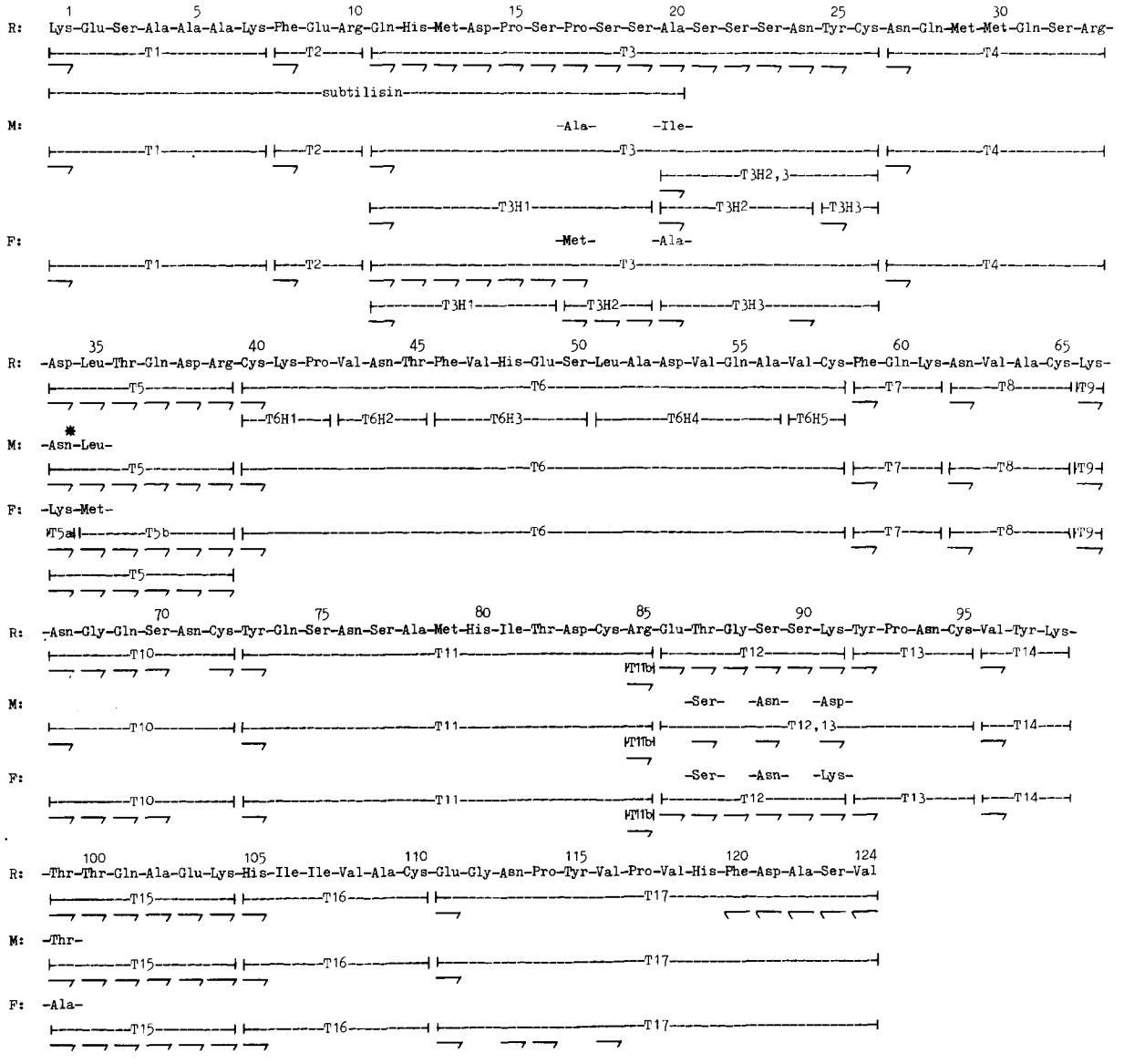

Fig.1. The primary structures of reindeer (R), moose (M) and fallow deer (F) pancreatic ribonucleases. T = peptides from the tryptic digests. TH = peptides from the digestion of tryptic peptides with thermolysin. The reindeer *S*-peptide obtained from a subtilisin digest of native ribonuclease [14] also has been included in this Figure. (——→) Residues identified as their dansyl derivative. (←——) Residues identified by digestion with carboxypeptidase A. (Asn*) Asparagine with carbohydrate.

from reindeer ribonuclease after a single Edman degradation cycle, which still had a free amino group (table 4).

The presence of aspartic acid at position 34 in reindeer ribonuclease was also evident from the low mobility of peptide T5 during paper electrophoresis at pH 3.5 and the blue colour obtained after staining with ninhydrin. (The homologous peptide from roe deer ribonuclease A with N-terminal asparagine gives a yellow colour with ninhydrin.) As can be seen from

Table 2
The amino acid composition of peptides obtained after secondary cleavage of tryptic peptides with thermolysin

	MOOSE				FALLOW DEER			REINDEER				
	T3H1	T3H2	T3H3	T3H2,3	T3H1	T3H2	T3H3	T6H1	T6H2	T6H3	T6H4	T6H5
Asp	1.2(1)	1.0(1)		1.2(1)	1.2(1)		1.1(1)		1.0(1)		1.0(1)	
Thr									0.9(1)			
Ser	3.1(3)	2.2(3)		2.6(3)	1.0(1)	2.1(2)	3.2(3)			1.1(1)		
Glu	0.9(1)				1.0(1)					1.1(1)	1.0(1)	
Pro	1.5(1)				1.0(1)			1.0(1)				
Ala	0.9(1)						0.9(1)				1.9(2)	
Val									1.0(1)	0.9(1)	1.2(1)	1.0(1)
Met	0.9(1)				0.6(1)	1.1(1)						
Ile		1.0(1)		1.2(1)								
Leu											0.9(1)	
Tyr			1.0(1)	1.0(1)			1.0(1)					
Phe										0.9(1)		
Lys								1.0(1)				
AetCys			+ (1)	+ (1)			+ (1)	+ (1)				+ (1)
His	+ (1)				+ (1)					1.0(1)		
nmoles	17	10	5	9	12	21	19	250	220	300	450	350
position in seq.	11-19	20-24	25-26	20-26	11-16	17-19	20-26	40-42	43-45	46-50	51-56	57-58

fig.1 the requirement for carbohydrate attachment (the presence of an Asn–X–Ser/Thr sequence) is only met at position 34–36 in moose ribonuclease.

Due to shortage of material the charge of peptide T12, 13 from moose ribonuclease could not be determined. From its low mobility at pH 3.5 we concluded that there is one aspartic acid in this peptide and we tentatively assigned this free carboxylic group by homology to residue 91 and not to residues 89 or 94. So far, substitution of residue 91 has been observed only in coypu ribonuclease, where also a substitution of lysine by aspartic acid was found [13].

Table 3
The carbohydrate composition of glycopeptide T5 of moose ribonuclease

Fucose	0.8
Mannose	3.3
Galactose	1.3
N-acetylglucosamine	2.0
Sialic acid	0.7

The amounts of monosaccharide are given in nmoles per nmole peptide. However, it is possible that part of peptide T5 was lost between amino acid and carbohydrate analysis.

The deer ribonucleases clearly constitute a group of related sequences. Fallow deer ribonuclease only differs at position 17 from red deer ribonuclease [3,4]. Roe deer, reindeer and moose ribonuclease differ in more positions, but also form a group of related sequences. Including the polymorphism at position 64 in roe deer ribonuclease [3] there are 9 positions in which the five deer ribonucleases show sequence differences (fig.1).

Table 4
Amide assignments

Peptide	Species	Number of Edman degradation cycles performed	Charge	Conclusion
T3	R	–	+1	Gln-11
	R	1	0	Asp-14
	R	2	−1	Asn-24
	R	4	0	
T5	R	–	−1	Asp-34 (R)
	F	–	+1	Gln-37
	R,F	2	0	Asp-38
	R,F	4	0	
T6H4	R	–	−1	Asp-53
	R	3	0	Gln-55
T11	R,M,F	–	+2	Gln-74
	F	1	+1	Asn-76
	F	2	+1	Asp-83
	F	4	+1	
T12	R,F	–	0	Glu-86 (R)
	R	1	+1	Glu-86 (F)
T12	F	2	0	Asn-89 (F)
	F	4	0	
T15	R,F	–	0	Gln-101
	R,F	3	−1	Glu-103
T17	R	–	−1	Glu-111
	R	1	0	Asn-113
	R	3	0	

Charges of peptides are derived from the electrophoretic mobility at pH 6.5.
(R) Reindeer; (M) Moose; (F) Fallow deer.

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