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QUARTERLY

Primary structure of porcine spleen ribonuclease: sequence homology*

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Key words: ribonuclease; porcine spleen; primary structure

The primary structure of porcine spleen RNase (RNase Psp1) was investigated as a mean of assessing the structure-function relationship of base non-specific ribonucleases of animal origin. N-terminal analysis of RNase Psp1 yielded three N-terminal sequences. These peptides were separated by gel-filtration on Superdex 75HR, after reduction and S-carboxymethylation of RNase Psp1. Determination of the amino-acid sequence of these peptides indicated that the RNase Psp1 preparation consisted of three peptides having 20 (RCM RNase Psp1 pep1), 15 (RCM RNase Psp1 pep2), and 164 (RCM RNase Psp1 pro) amino-acid residues, respectively. It possessed two unique segments containing most of the active site amino-acid residues of the RNases of the RNase T2 family. The alignment of these three peptides in RNase Psp1 was determined by comparison with the other enzymes in the RNase T2 family. The overall results showed that RCM RNase Psp1 pep1 and RCM RNase Psp1 pep2 are derived from the N-terminal and C-terminal regions of RNase Psp1, respectively, probably by processing by some protease. The molecular mass of the protein moiety of RNase Psp1 was 23235 Da.

Porcine spleen RNase has been purified by Bernardi & Bernardi [1] and has a pH-optimum of 5.0. Similar enzymes have also been purified by Maever & Greco [2] and Delaney [3] from bovine and human spleen, respectively. We isolated a similar RNase from bovine spleen (RNase Bsp1) [4] and elucidated its primary structure [5]. The structure of bovine spleen RNase possesses two segments that include amino-acid residues important for its enzymatic activity and commonly observed in RNase T2 family RNases [6]. In this study, we investigated the primary structure of an acid RNase from porcine spleen (RNase Psp1) and compare it with homologous RNases from other animals, in

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Abbreviations: PTH, phenylthiocarbamoylhydantoin; RNase Psp1, an acid ribonuclease from porcine spleen; RCM RNase Psp1, reduced and S-carboxymethylated RNase Psp1; RCM RNase Psp1 pro, pep1 and pep2, fragments produced by reduction and S-carboxymethylation of RNase Psp1.

order to obtain further information on the structure-function relationship of base non-specific acid RNases.

MATERIALS AND METHODS

Enzymes. Porcine spleen RNase Psp1 was purified from porcine spleen essentially according to the procedure of Ohgi *et al.* [4], with some minor modification. RNase Psp1 yielded a single band in SDS/PAGE. The molecular mass estimated by SDS/PAGE with the aid of a Promega molecular mass kit (Promega, Madison, WI, U.S.A.) was about 29 kDa. Lysyl endopeptidase (EC. 3.4.21.50) was a product of Seikagaku Kogyo (Tokyo). α-Chymotrypsin and trypsin were purchased from Sigma (St. Louis, MO., U.S.A.).

SDS/polyacrylamide gel electrophoresis. SDS/PAGE was performed in 12.5% polyacrylamide gel by the procedure of Laemmli [7], and the gel was stained with Coomassie brilliant blue.

Amino-acid analysis. Amino-acid analysis was performed with AccqTag amino acid analysis system (Millipore Japan, Tokyo). Tryptophan content was determined by the method of Pajot [8].

Hexosamine. Hexosamine content was measured with the AccqTag amino acid analysis system after hydrolysis in 4 N hydrochloric acid at 100°C for 5 h.

Preparation of RCM RNase Psp1. RNase Psp1 was reduced and S-carboxymethylated as described by Crestfield *et al.* [9]. The reduced and S-carboxymethylated RNase Psp1 (RCM RNase Psp1) was separated from excess reagents by gel-filtration on FPLC Superdex 75HR 10/30 and Sephadex G-75 equilibrated with 50 mM trimethylamine acetate buffer (pH 8.0).

Preparation of maleylated RNase Psp1. RNase Psp1 was maleylated as described by Butler *et al.* [10].

Protease digestion. The protein was digested with lysyl endopeptidase, trypsin, and α-chymotrypsin in 100 mM trimethylamine acetate buffer (pH 8.0). Protein was added to an enzyme-substrate ratio of 1:200 (w/w), and digested at 37°C for 5 h.

Fractionation of peptides by reversed phase HPLC. Fractionation of peptides by HPLC was performed with a TSK ODS 80 column (4 mm × 250 mm) (Tohso, Tokyo) and a Capcell pak C-18 column (10 mm × 250 mm) (Shiseido, Tokyo). The TSK ODS 80 column was equilibrated with 0.1% trifluoroacetic acid, and the peptides were eluted with a linear gradient of acetonitrile up to 60%. The flow rate was 1 ml/min. A Capcell pak C-18 column equilibrated with 40 mM trimethylamine acetate buffer (pH 8.0) was eluted with a linear gradient of acetonitrile up to 60%. The flow rate was 3 ml/min.

Fractionation of protein by Superdex 75. Gel-filtration on a Superdex 75 HR 10/30 column (1 mm × 30 cm), equilibrated with 50 mM trimethylamine acetate buffer (pH 7.5), was performed at 22°C at a flow rate of 1 ml/min.

Peptide nomenclature. L and C stand for lysyl endopeptidase- and α-chymotrypsin-digested peptides, respectively. The peptides are numbered in order from the N-terminus to the C-terminus. MT stands for the peptides obtained by trypsin digestion of maleylated RCM RNase Psp1.

RESULTS

Preparation of RNase Psp1

The RNase Psp1 prepared yielded a single band on SDS/PAGE (Fig. 1). Its molecular mass estimated by electrophoresis in the absence of reducing agent was approx. 29 kDa, but that in the presence of reducing agent it was about 24 kDa.

Primary structure of RNase Psp1

The N-terminal of RNase Psp1 was measured by Edman degradation to identify the primary structure of RNase Psp1. The results showed the presence of three peptides in this preparation (Table 1). The reduced and S-carboxymethylated RNase Psp1, prepared according to the method of Crestfield *et al.* [9], was fractionated by Superdex gel-

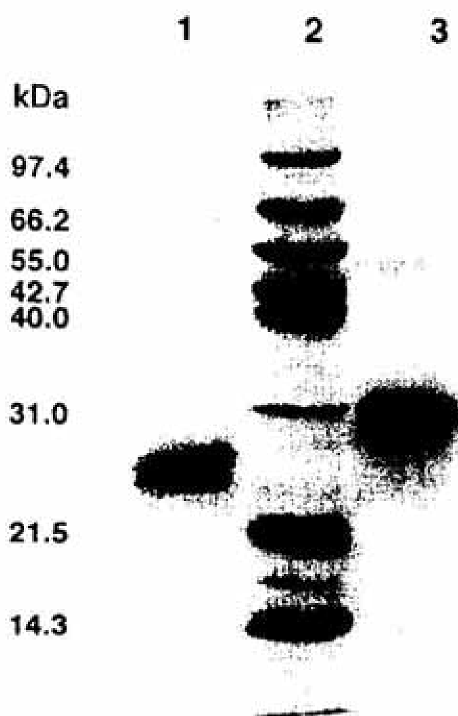


Figure. 1. Electrophoresis of RNase Psp1.

SDS/PAGE in 12.5% polyacrylamide gel. Lane 1, RNase Psp1 in the presence of 2-mercaptoethanol; lane 2, standard marker proteins; lane 3, RNase Psp1 in the absence of 2-mercaptoethanol.

filtration and yielded the RCM derivatives of three peptides, pep1, pep2, and pro (Fig. 2). The results of Edman degradation of these fractions are shown in Fig. 3 and Table 1. The amino-acid composition of these three fractions is shown in Table 2.

RCM RNase Psp1 pep1 and RCM RNase Psp1 pep2 consisted of 20 and 16 amino-acid residues, respectively. The N-terminal amino-acid sequence of RCM RNase Psp1 pro obtained by subtraction of the two peptides, RCM RNase Psp pep1 and 2, from that of native RNase Psp1 was identical to that of RCM RNase Psp1 pro. Since there is no free SH group in RNase Psp1, and RCM RNase Psp1 pep1 and RCM RNase Psp1 pep2 each contain a half cystine residue, these two peptides are probably connected by a disulfide bridge with the RCM RNase Psp1 pro fraction. RCM RNase Psp1 was separated from excess reagent by Sephadex G-75 and digested with α-chymotrypsin. The α-chymotryptic digest was fractionated into 10 major peaks (Fig. 4). The amino-acid sequences of these 10 peptides are shown in Fig. 3. The sequences of C1 and C10 are identical to those of RCM RNase Psp1 pep1 and pep2, respectively (Fig. 3). The sequence of C2 is present in the N-terminal sequence of RCM RNase Psp1 pro determined up to the 21st residue.

The finding that the N-terminal sequence of RNase Psp1 consists of three peptides is explained by the combination of C1, C2, and C10. Similarly, RCM RNase Psp1 was digested by lysyl endopeptidase into 16 fractions (Fig. 5). The amino-acid sequences of these 16 peptides are shown in Fig. 3.

The sequences of L1 and L2 peptides were included in C1. The sequence of L3, L4, L5, L6 and C3 determined the N-terminal 65 amino-acid residues of RCM RNase Psp1 pro. The sequence of C5 connected L8, L9, L10, and L11. The sequence of L13 connected those of C6, C7 and C8. The sequence of L15 determined the alignment of C8 and C9. The RCM RNase Psp1 was maleylated and then treated with trypsin and the trifluoroacetic acid-soluble fraction of the tryptic digest fractionated similarly into four peaks (Fig. 6a). The acid-insoluble fraction was fractionated by Capcell pak C-18 column into two fractions (Fig. 6b). The amino-acid sequences of these fractions are shown in Fig. 3.

The amino-acid sequence of MT2 and MT3 determined the connection of L6 and L7 (L8), and of C5, C6, and C7, respectively. Thus the amino-acid sequence of the protein moiety consisting of 164 amino-acid residues was elucidated. The amino-acid sequence of C1

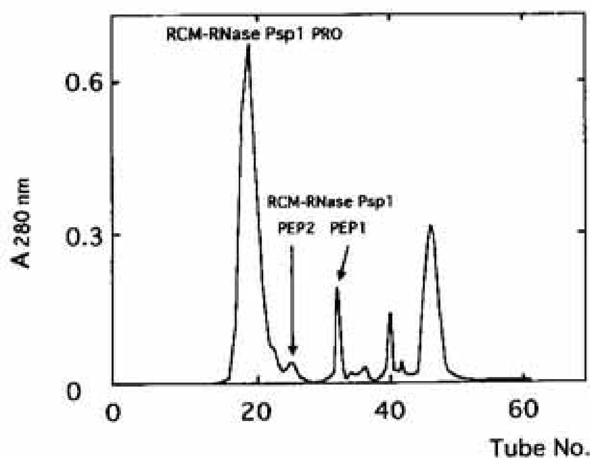


Figure 2. Gel-filtration of RCM RNase Psp1 preparation on a Superdex 75 HR 10/30 column.

Elution conditions are described in Materials and Methods.

Table 1. N-terminal amino-acid sequences of RNase Psp1, and the peptides, RCM RNase Psp1 pro, RCM RNase Psp1 pep1, and RCM RNase Psp1 pep2 (and approx. 3 nmol sample of each was applied)

Step	PTH-amino acid (pmol)					
	RNase Psp1			RCM RNase Psp1 peptides		
				Pro	pep1	pep2
1	L 1300	H 800	N 1300	N 1100	H 900	L 1050
2	E 2000			Cmc 1000	E 1100	E 900
3	I 1020	W 700	E 1000	E 920	W 800	I 820
4	K 840	H 600		H 520	K 890	Cmc 690
5	E 800	K 750	P 900	P 780	K 860	E 680
6	D 700	L 710	P 860	P 700	L 790	D 580
7	G 520	I 660	D 680	D 520	I 670	G 450
8	P 600	M 520	Y 600	Y 470	M 500	P 500
9	V 1140	W 420		W 480	V 580	V 470
10	F 500	H 370	T 280	T 320	H 440	F 420
11	Y 400	H 380	I 420	I 470	H 450	Y 320
12	P 380	W 410	H 300	H 290	W 400	P 300
13	P 810	G 300		G 350	P 410	P 350
14	P 270	M 280	L 320	L 360	M 370	P 260
15	K 120	T 100	W 180	W 300	T 200	K 150
16	E 140	V 240	P 350	P 270	V 330	E 100
17	D 270			D 230	Cmc 250	
18	N 180	K 180		K 180	N 200	
19	E 140	S 100		S 120	E 160	
20	K 100	G 110		G 180	K 120	
21	E 100			E 90		

peptide is very similar to the N-terminal sequence of RNase Oy from the oyster [11], drosophila RNase [12], RNase Bsp1 from bovine spleen [5], chicken liver RNase [13] and bullfrog RNase [14], as shown in Fig. 7. The amino-acid sequences of C10, L16, and MT-5 were also very similar as those of the C-terminal sequence of the other RNases described above, and the alignments of RNase Psp1 pep1, RCM RNase Psp1 pep2, and RCM RNase Psp1 pro were estimated as shown in Fig. 3.

There was one unidentified amino acid in each of the amino-acid sequence of L4, L5, L6, L11, C3, C4, C5, MT1, and MT3.

Based on the following evidence, we concluded that these positions were occupied by Asn residues: (i) RNase Psp1 is a glycoprotein (see Table 2), (ii) the sequence coincided with the consensus sequence for the N-glycoside

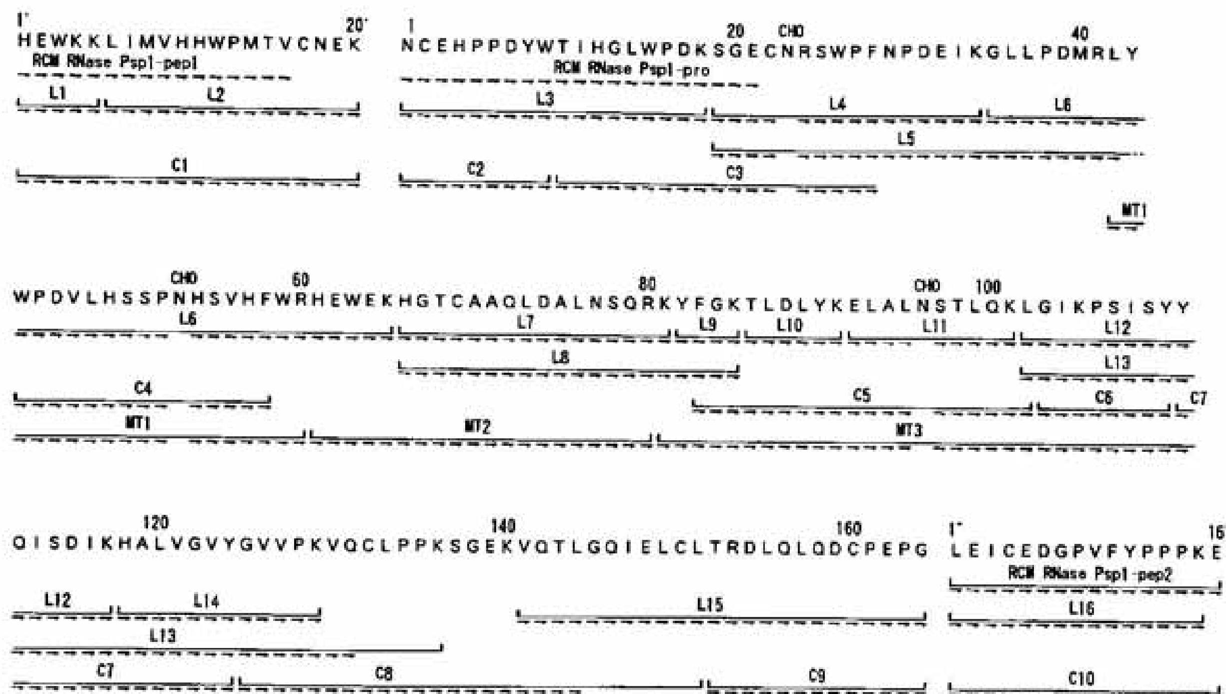


Figure 3. Amino-acid sequence of RCM RNase Psp1.

The alignment of peptides is indicated as follows: The arrows designate amino-acid sequence determined by Edman degradation. C and L mean digestion products with α-chymotrypsin, and lysyl endopeptidase, respectively. MT means tryptic peptide of maleylated RCM RNase Psp1. Peptides are numbered from the N-terminus to the C-terminus. The lines with the vertical bars at both ends indicate regions where the sequence was estimated from the results of amino-acid analysis. Numbering of the RCM RNase Psp1 pep1 and pep2 is indicated by prime (') and double prime (''), respectively.

junction in glycoprotein, and (iii) these peptides contain one more Asp residue than expected from the determined sequence (data not shown).

The amino-acid composition of RNase Psp1, shown in Table 2, is practically identical to that of the sum of the protein portions of RNase Psp1, C1, and C10. The molecular mass of the protein moiety of RNase Psp1 was at least 23135 (200 amino-acid residues). Comparison of the amino-acid sequence with those of other animal RNases belonging to the RNase T2 family suggests that the number of deleted amino-acid residues between RNase Psp1 pro and pep1 and pep2 is probably zero and no more than two, respectively.

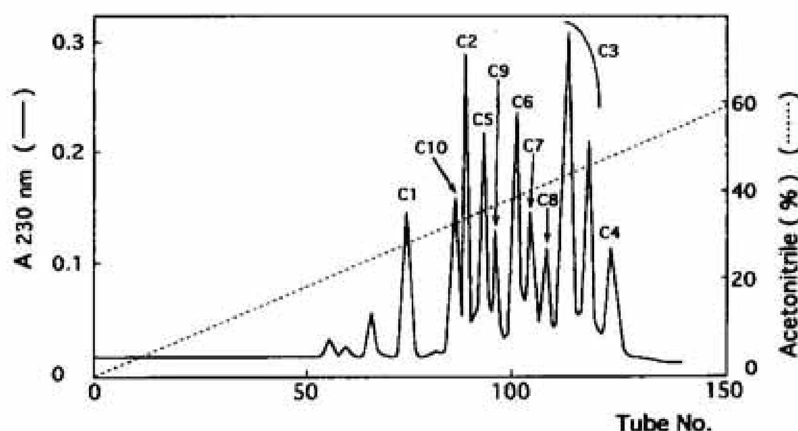


Figure 4. Fractionation of the α-chymotryptic digest of RCM RNase Psp1 by reversed-phase HPLC.

The α-chymotryptic digest of RCM RNase Psp1 was fractionated on a column of TSK ODS 80 (4 mm × 250 mm) equilibrated with 0.1% trifluoroacetic acid. The peptides were eluted with a linear gradient between the same buffer and 60% acetonitrile in 240 min. The flow rate was 1 ml/min.

Table 2. Amino-acid composition of RNase Psp1 and its peptides obtained by reduction and S-carboxymethylation of RNase Psp1.

	RNase Psp1 (Residue)		RCM RNase Psp1 peptides (Residue)					
	Experi-mental	Calcd. from the sequence	pro		pep1		pep2	
Asp	17.3	18	16.0	(16)	1.2	(1)	1.3	(1)
Glu	22.4	23	16.9	(18)	1.7	(2)	3.2	(3)
CM-Cys	7.4	8	5.8	(6)	0.7	(1)	0.9	(1)
Ser	10.6	11	11.2	(11)				
Gly	12.7	12	11.6	(11)			1.4	(1)
His	10.5	11	8.0	(8)	2.8	(3)		
Arg	5.0	5	5.4	(5)				
Thr	6.5	7	5.6	(6)	1.2	(1)		
Ala	5.5	5	5.4	(5)				
Pro	20.0	19	14.4	(14)	1.4	(1)	4.6	(4)
Tyr	7.5	8	6.7	(7)			1.3	(1)
Val	10.6	11	7.9	(8)		(2)	0.7	(1)
Met	3.2	3	1.2	(1)	2.0	(2)		
Ile	8.6	9	7.4	(7)	1.0	(1)	0.8	(1)
Leu	21.3	22	20.4	(20)	0.8	(1)	0.9	(1)
Phe	4.5	4	3.4	(3)			1.3	(1)
Lys	16.6	16	12.4	(12)	3.6	(3)	1.2	(1)
Trp	7.5	8	5.4	(6)	n.d.	(2)		
Total		200		164		20		16
GlcNAc	5.6							

Comparison of the primary structure of RNase Psp1 with the others T2 RNases

The primary structure of RNase Psp1 was compared with that of RNase T2 RNases (Fig. 7). RNase Psp1 has two segments which are important for the enzymatic activity of the other RNases in the RNase T2 family (the 44th–50th residue, and 101th–112th residue). The numbers of amino-acid residues of RNase Psp1 homlogous to those of bovine spleen RNase Bsp1 [5], chicken liver RNase CL1 [13], bullfrog RNase [14], drosophila RNase [12], and oyster RNase Oy [11] are 141, 110, 100, 65, and 65 residues, respectively, and RNase Psp1 very closely resembles RNase Bsp1 [5] among animal RNases. Accordingly we were able to conclude that RNase Psp1 is a typical RNase of the RNase T2 family.

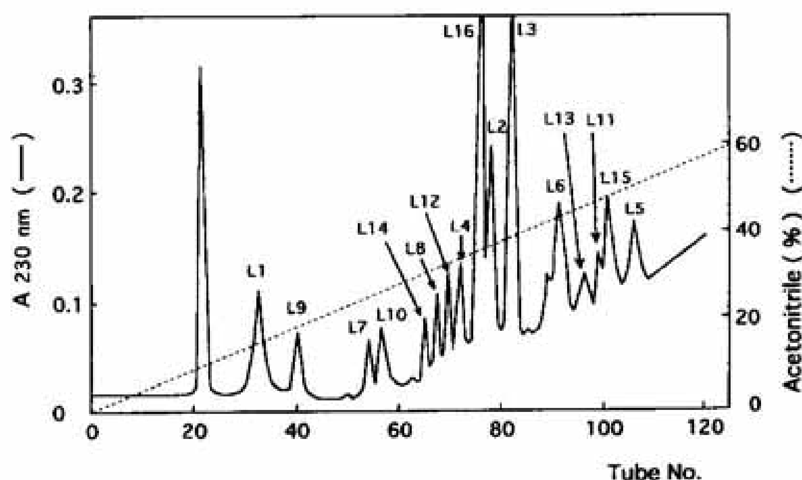
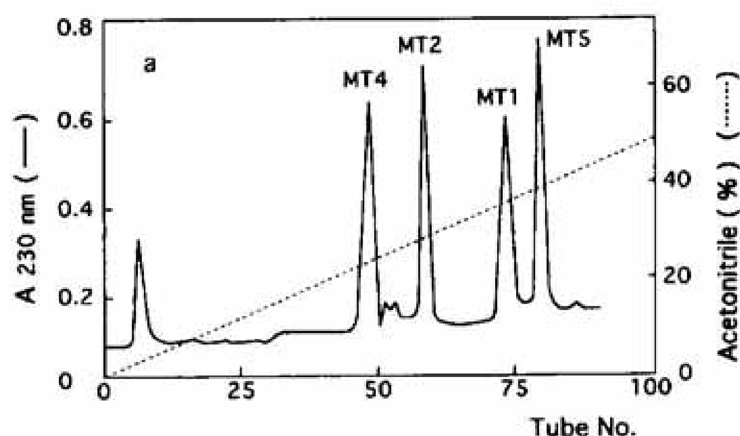


Figure 5. Fractionation of lysyl endopeptidase digest of RCM RNase Psp1 by reversed-phase HPLC.

The lysyl endopeptidase digest of RCM RNase Psp1 was fractionated on a TSK ODS 80 column, as described in Fig. 4.



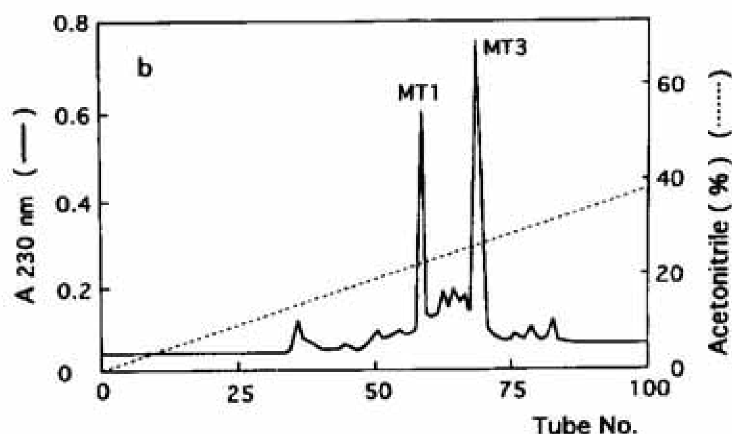


Figure 6. Fractionation of the tryptic digest of maleylated RCM RNase Psp1 by reversed-phase HPLC.

(a) The trifluoroacetic acid-soluble fraction of the tryptic digest of maleylated RNase Psp1 was fractionated on a column of TSK ODS 80, as described in Fig. 4. (b) The trifluoroacetic acid-insoluble fraction of the tryptic digest of maleylated RCM RNase Psp1 was fractionated on a Capcel pak C-18 column, as described in Materials and Methods

DISCUSSION

Amino-acid sequencing of RNase Psp1 showed that all the amino- acid residues comprising the active site of RNase Rh from *Rhizopus niveus* [6] (His-46, His-104, His-109, Glu-105, and Lys-108) are conserved in RNase Psp1. Among the amino-acid residues comprising the major base recognition site of RNase Rh (Asp-51, Trp-49, and Tyr-57), Asp-51 and Trp-49 are conserved, but Tyr-57 is not. This lack of Tyr-57 (or Leu in some plants) seems to be a common phenomenon in animal RNases [11–14], but not in plant and fungal RNases [6]. Among the constituents of the minor base recognition site of RNase Rh (B2 site, Gln-32, Pro-92, Ser-93, Asn-94, Gln-95 and Phe-101)**, only Phe-101

**Nakamura, T.K., Ishikawa, N., Hamashima, M., Kurihara, H., Nonaka, T., Mitsui, Y., Ohgi, K. & Irie, M. The 3rd International Meeting on Ribonucleases, Chemistry, Biology and Biotechnology (Capri, Italy, May, 1993).

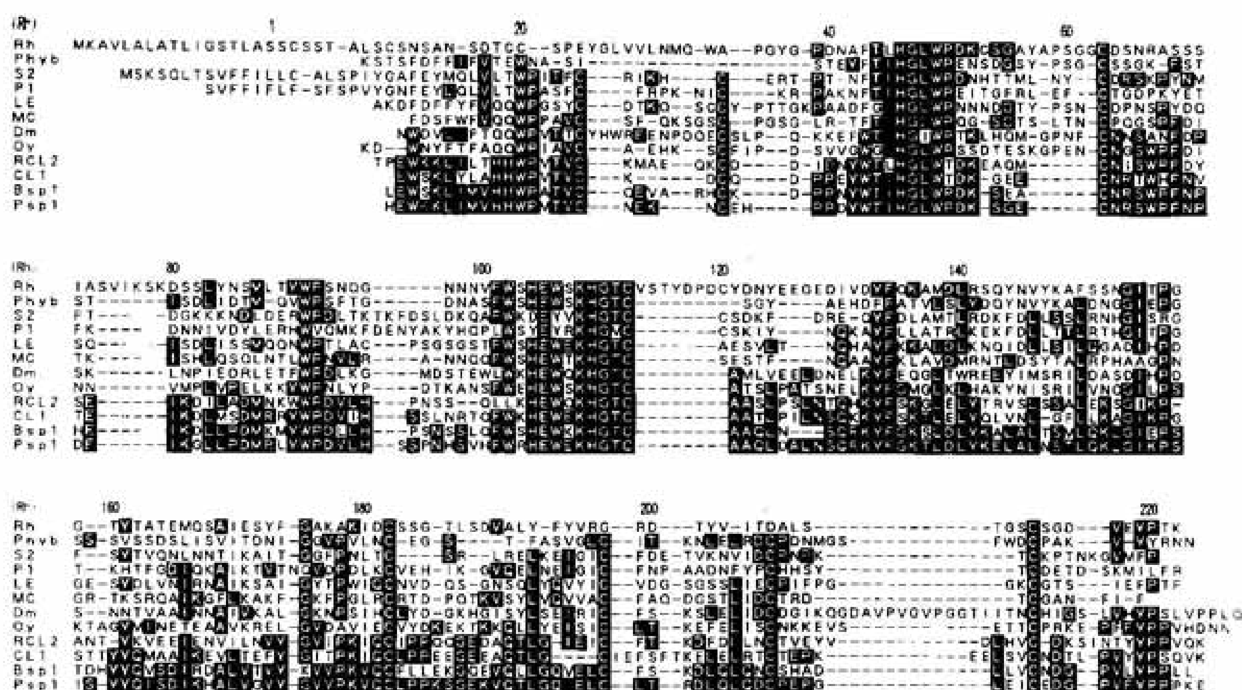


Figure 7. Comparison of the amino-acid sequence of RNases belonging to the RNase T2 family.

Rh, RNase Rh from *R. niveus*; Phyb, RNase Phyb from *P. polycephalum* [15]; S2, a self-incompatibility factor of *Nicotiana alata* [16]; P1, a self-incompatibility factor of *Petunia inflata* [17]; Le, RNase LE from cultured tomato (*Lycopersicon esculentum*) [18]; MC, RNase from bitter gourd (*Momordica charntia*) [19]; Dm, RNase from *Drosophila melanogaster*) [12]; Oy , RNase Oy from oyster [11]; RCL2, bullfrog RNase RCL2 (*Rana catesbeiana*) [14]; CL1, chicken liver RNase [13]; Bsp1, RNase from bovine spleen [5]; Psp1, RNase from porcine spleen.

and Pro-92 are conserved in RNase Psp1. The others, Ser-93, Asn-94, Gln-95 are replaced by Asp, Val, and Leu, respectively. Gln-32 may be replaced by His or Glu, but, it is not possible to identify which corresponds to Gln-32 of RNase Rh, because we have no precise knowledge of the three-dimensional structure of RNase Psp1. We compared its primary structure with that of the other animal RNases, as shown in Fig. 7. RNase Psp1 most closely resembles RNase Bsp1 [5]. The amino-acid residues homologous with those of RNase Bsp1, RNase CL1, and RNase RCL2, expressed per 100 amino-acid residues, are 70, 58 and 50 residues, respectively. A similar comparison with secretory RNase (RNase A family RNase) was performed. The amino-acid residues of porcine pancreatic RNase homologous to bovine pancreatic RNase A [20], chicken liver RNase CL2 [21], and bullfrog liver RNase [22], and egg RNase [23] are 77, 33, 27 and 27 amino-acid residues, respectivly. To compare the rate of evolutionary change in the RNase T2 family and the RNase A family, the frequency of homologous amino-acid sequences in porcine RNase and bovine, chicken, and bullfrog RNases for both RNase A and T2 families have been plotted in Fig. 8. These comparisons showed that the correlation coefficient between the evolutionary rates of both families are 1.06 (R^2 = 0.88), and not very much different each other, although this is not conclusive, because relatively few data are available at present.

When we compared the N-terminal portions of RNase T2 family RNases, we found that the sequences are finger printing-like zones as shown in Fig. 9. Bacteria and amoeba RNases have the sequences shown as (a) and (b), and plant RNases and self-incompatibility factors of plants have the sequences (c) and (d), respectively. Lower and higher animals have sequence (e) and (f), respectively. In this figure, the last WP sequence is almost completely conserved in plants and animals, but not in bacteria and amoeba. However, some of the other sequences seem to change, by evolution, e.g., first F mutates to W (also aromatic amino-acid residue), the 8 and 9th

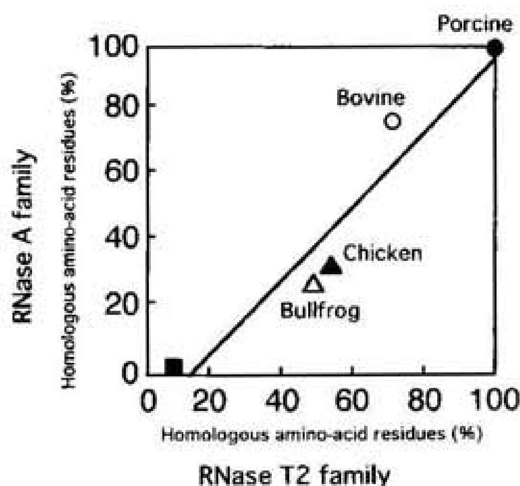


Figure 8. Correlation between the frequence of homologous amino acid residues in porcine RNase and other RNases (chicken, bovine spleen and porcine spleen and bullfrog).

The numbers along the ordinate and abscissa are numbers of homologous amino acids per 100 amino-acid residues. Three amino-acid residues conserved (His-12, His-119, and Lys-41 (in RNase A numbering) in the RNase A family, and 14 common amino-acid residues conserved in the RNase T2 family, have been excluded from the calculations of the homologous amino-acid residues.

QQ sequence to HH in higher animals. The other amino-acid residues mutate depending to organism in some limited range, the 6th F mutates L to L or M. The amino-acid sequence of RNase Psp1 in this region was WKKLIMVHHWP, and closely coincided with those of animal RNases.

De Duve *et al.* [26] showed that acid RNase is associated with lysosomal fraction in rat liver tissues. Investigations on the localization of acid RNase by zonal centrifugation [27] and sucrose gradient centrifugation [28] showed that acid RNase is located in the lysosome-like fraction but not in the fraction containing acid phosphatase. By contrast, Baudhuin *et al.* [29] showed by isopicnic centrifugation that acid RNase and acid phosphatase are in the same fraction. In addition to these studies, Futai *et al.* [30] partially purified acid RNase with a pH optimum at 5.8 from rat liver and the lysosome fraction of rat liver, and claimed that acid RNase is a lysosomal enzyme. The results in this paper and those of Bernardi & Bernardi [1], as well as our previous results on bovine spleen acid RNase [4, 5], show that these RNases are only one or at least the major species of acid RNase in these tissues and they have a very sharp optima at around pH 5–6 like rat liver acid RNase [30]. The primary structure of rat liver RNase has not yet been determined. However, from the overall results, it is highly probable that RNase Bsp1 and RNase Psp1 are lysosomal or lysosomal-like particle RNases.

			15	16				20				
(a)	**F**	**D**	y	**Y**	a	m	**A**	**L**	**S**	**W**	S	**bacteria**
			r		v	l					q	
(b)	**F**	**D**	**F**	**F**	**I**	**F**	**V**	**T**	**E**	**W**	**N**	**amoeba**
(c)	**F**	**D**	F	**F**	Y	F	V	Q	**Q**	**W**	**P**	**plant**
			y		w	l	s	l				
			s		a							
(d)	**F**	d	Y	f	Q	**L**	**V**	L	T	**W**	**P**	**plant (self-incompatibility factors)**
		e	l	l	e			e	q			
			h	l	k							
				m								
				w								
(e)	**W**	d	y	f	l	**F**	t	**Q**	**Q**	**W**	P	**invertebrate**
		n	v	l	f		a					
(f)	**W**	s	**K**	**L**	l	m	v	**H**	**H**	**W**	**P**	**vertebrate**
		k			y	l	a					

Figure 9. Changes in the N-terminal portion of the sequence from plants through lower animals and vertebrates.

Bold capital letters: conserved sequences in the subgroup; capital letters, conserved at high frequency; lower case letters, conserved less than 40%. The numbering at the top of the sequence is RNase Rh numbering. The sequence of (a) bacteria [24, 25]; (b) amoeba RNase (*F. polycephalum*); (c) plant RNases; (d) self-incompatibility factors; (e) *Drosophila* and oyster RNases; (f) RNases from chicken, bovine and porcine spleen.

REFERENCES

1. Bernardi, A. & Bernardi, G. (1966) Studies on acid hydrolases. III. The isolation and proper-

ties of spleen acid ribonuclease. *Biochim. Biophys. Acta* **129**, 23–31.

2. Maever, M.E. & Greco, A.E. (1952) The chromatographic separation and characterization of the acid and an alkaline ribonuclease of bovine spleen and liver. *J. Biol. Chem.* **237**, 736–741.

3. Delaney, R. (1963) Chemical, physical, enzymatic properties of several human ribonucleases. *Biochemistry* **2**, 438–444.

4. Ohgi, K., Sanda, A., Takizawa, Y. & Irie, M. (1988) Purification of acid ribonuclease from bovine spleen. *J. Biochem.* **103**, 267–273.

5. Narumi, H., Ogawa, Y., Iwama, M., Kusano, A., Sanda, A., Ohgi, K. & Irie, M. (1997) Bovine spleen acid ribonuclease is a member of the RNase T2 family. *J. Biochem Mol. Biophys.* **1**, 21–26.

6. Irie, M. (1997) RNase T1/RNase T2 family RNases; in *Ribonucleases* (D'Alessio, G. & Riordan, J.F., eds.) pp. 101–130, Academic Press, New York.

7. Laemmli, U.K.(1970) Cleavage of structural proteins during the assembly of the bacteriophage T. *Nature* **227**, 680–685.

8. Pajot, P. (1986) Fluorecence of protein in 5 M guanidine Hydrochloride. *Eur. J. Biochem.* **63**, 263–269.

9. Crestfield, A., Moore, S. & Stein, W. (1963) The preparation and enzymatic hydrolysis of reduced and S-carboxymethylated protein. *J. Biol. Chem.* **238**, 622–627.

10. Butler, P.J.G., Harries, J.L. & Hartley, B.S. & Leberman, R. (1969) The use of maleic anhydride for the reversible blocking of amino groups in polypeptide chains. *Biochem. J.* **112**, 629–689.

11. Watanabe, H., Narumi, H., Inaba, T., Ohgi, K. & Irie, M. (1993) Purification, some properties, and primary structure of a base non-specific ribonuclease from oyster (*Crassostrea gigas*). *J. Biochem.* **114**, 800–807.

12. Hime, G., Prior, L. & Saint, R. (1995) The *Drosophila melanogaster* genome contains a member of the Rh/S-glycoprotein family of ribonuclease-encoding genes. *Gene* **158**, 203–207.

13. Uchida, T., Hayano, K., Iwama, M., Watanabe, H., Sanda, A., Ohgi, K. & Irie, M. (1996) Base specificity and primary structure of so-called poly U preferential ribonuclease from chicken liver. *Biosci. Biotech. Biochem.* **60**, 1982– 1988.

14. Inokuchi, N., Kobayashi, H., Miyamoto, N., Koyama, T., Iwama, M., Ohgi, K. & Irie, M. (1997) Primary structure of base non-specific and acid ribonuclease from bullfrog (*Rana catesbeiana*). *Biol. Pharm. Bull.* **20**, 471–478.

15. Inokuchi, N., Koyama, T., Sawada, F. & Irie, M. (1993) Purification, some properties and primary structure of base non-specific ribonuclease from *Physarum polycephalum*. *J. Biochem.* **113**, 425–432.

16. MaClure, B.A., Haring, V., Evert, P.R., Anderson, M.A., Simpson, R.J., Sakiyama, F. & Clarke, A.E. (1989) Style self-incompatibility gene products of *Nicotiana alata* as ribonuclease. *Nature* **342**, 955–958.

17. Ai, Y., Singh, A., Coleman, C.E., Ioerger, T.R., Kheyer-Pour, A. & Kao, T.-H. (1990) Self incompatibility in *Petunia inflata*. Isolation and charcterization of DNAs encoding three S-allele associated proteins. *Sex Plant Reprod.* **3**, 130–138.

18. Jost, W., Bak, H., Glund, K., Terpstra, P. & Beintema, J.J. (1991) Amino acid sequence of an extra-cellular phosphate starvation induced ribonuclease from cultured tomato, (*Lycopersicon esculentum*). *Eur. J. Biochem.* **198**, 1–6.

19. Ide, H., Kimura, M., Arai, M. & Funatsu, G. (1991) The complete amino acid sequence of ribonucleae from the seeds of bitter gourd (*Momordica charantia*). *FEBS Lett.* **284**, 161–164.

20. Richards, F.M. & Wychoff, H.W. (1971) Bovine pancreatic ribonuclease; in *The Enzymes* (Boyer, P.D., ed.) vol. 4, 3rd ed., pp. 646–806, Academic Press, New York.

21. Hayano, K., Iwama, M., Sakamoto, H., Watanabe, H., Sanda, A., Ohgi, K. & Irie, M. (1993) Characterization of poly C preferential ribonucleases from chicken liver. *J. Biochem.* **114**, 156–162.

22. Nitta, R., Katayama, N., Okabe, Y., Iwama, M., Watanabe, H., Abe, Y., Okazaki, T., Ohgi, K. & Irie, M. (1989) Primary structure of a ribonuclease from bullfrog (*Rana catesbeiana*). *J. Biochem.* **106**, 729–735.

23. Titani, K., Takio, K., Kuwada, M., Nitta, K., Sakakibara, F., Kawauchi, H., Takayanagi, G., Hakomori, S. (1988) Amino acid sequence of sialic acid binding lectin from frog (*Rana catesbeiana*). *Biochemistry* **26**, 2189–2194.

24. Meador, J., III. & Kennel, D. (1990) Cloning and sequencing the gene encoding *Escherichia coli* ribonuclease I: Exact physical mapping using genome library. *Gene* **95**, 1–7.

25. Favre, D., Philip, K. & Timmis, K.N. (1993) Relatedness of a periplasma, broad-specificity RNase from *Aeromonas hydrophila* to RNase I of *Escherichia coli* and to a family of eukaryotic RNases. *J. Bacteriol.* **175**, 3710–3722.

26. de Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. & Appelman, F. (1955) Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat liver tissue. *Biochem. J.* **60**, 604–617.

27. Rahman, Y.E., Howe, J.F., Nance, S.L. & Thomas, J.F. (1967) Studies on rat liver ribonuclese. II. Zonal centrifugation of acid ribonuclease; Implication for the heterogeneity of lysosome. *Biochim. Biophys. Acta* **146**, 484–492.

28. Rahman, Y.E. & Cerny, E.A. (1969) Studies on rat liver ribonucleases. III. Further studies on heterogeneity of liver lysosomes. Intracellular localization of acid ribonuclease and acid phosphatase in rats of various age. *Biochim. Biophys. Acta* **178**, 61–67.

29. Baudhuin, P., Peters-Joris, C. & Bartholeyns, J. (1975) Hepatic nucleases. 2. Association of polyadenylase, alkaline ribonuclease and deoxyribonuclease with rat-liver mitochondria. *Eur. J. Biochem.* **57**, 213–220.

30. Futai, M., Miyata, S. & Mizuno, D. (1969) Acid ribonuclease of lysosomal and soluble fractions from rat liver. *J. Biol. Cnem.* **244**, 4951–4960.