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Human extracellular ribonucleases: multiplicity, molecular diversity and catalytic properties of the major RNase types

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Abstract. Human extracellular ribonucleases (RNase), together with other members of the mammalian RNase A superfamily, can be classified into four different RNase families on the basis of their structural, catalytic and/or biological properties. Their occurrence and main distinctive features have been described, and the information available on their catalytic properties has been analysed and discussed in comparison with those of other animal RNases. On the basis of some results obtained with various single- and double-stranded polyribonucleotides, it has been proposed that while pancreatic-type (pt) RNases could be defined as single-strand/pyrimidine 'preferring' ribonucleases, mammalian nonpancreatic-type (npt) RNases may be referred to as single-strand/pyrimidine 'specific' ribonucleases. In addition, some data concerning human nptRNases may support the suggestion [Cuchillo et al. (1993) FEBS Lett. **333:** 207–210] that the enzyme 'ribonuclease' should be reclassified as 'transferase'.

Key words. Human ribonuclease(s); RNase superfamily; neurotoxin(s); EDN; ECP; angiogenin.

Introduction

Investigations on ribonuclease activities present in human body fluids and tissues date back 45 years, and the great deal of study on them (from 1952 to 1979) has been reviewed by Levy and Karpetsky [1]. The first reports gave little information on the nature of the enzyme being examined and, apart from the important preliminary studies of Delaney [2] and Ukita et al. [3], the biochemical and immunological characterization of human RNases was largely an effort of the last 5 years of the 1970s [1]. These studies led to the definition of two major classes [4] of human ribonucleases: the 'secretory' or plasma/pancreas type and the 'nonsecretory' or liver/spleen type, which were shown to possess different catalytic and antigenic properties [1]. During the late 1970s, in addition to an inherent interest in the enzymes themselves, the study of human ribonucleases was stimulated by suggestions that these enzymes, when released into circulation or excreted in urine, might serve as diagnostic markers for a variety of diseases [1]. But the multiplicity of these enzymes [5] made it difficult to prove any correlation between RNase activity and disease states. In particular, in the early 1980s, the validity of any conclusions based on enzymatic determinations of RNase activity in body fluids was questioned [6]. In addition, it was concluded that high circulating levels of both the so-called secretory and nonsecretory RNases (measured by radio immunological assay (RIA) procedures) were primarily related to kidney malfunction, and no relationship with cancer or any other specific disease was found [6–8].

In the mid 1980s, human RNases gained new interest when a series of initially unrelated studies linked several functionally diverse proteins to the RNase A superfamily [9] on the basis of their primary structure. Further investigations have indicated that these proteins belong to a group of RNases that possess special biological actions correlated with their RNase activity (reviewed in refs. 10–12) such as neurotoxicity, angiogenic activity, immunosuppressivity and antitumour activity.

However, the physiological roles of nondigestive extracellular ribonucleases found in fluids and tissues other than pancreas are to date poorly understood.
In this article the information available on the main structural and catalytic properties of the major human extracellular RNases (together with those of other animal ribonucleases) will be comparatively analysed and discussed.

Classification, occurrence and features

Human extracellular ribonucleases are widely distributed in various organs and body fluids and, together with other members of the mammalian RNase A superfamily, have been recently grouped into four different RNase families [13] on the basis of their structural, catalytic and/or biological characteristics. According to this classification [13] human ribonucleases not only found in pancreas but also in other tissues and fluids (characterized by showing sequences as well as stuctural and catalytic properties similar to those of bovine [14] or human [15–17] pancreatic RNases) belong to the mammalian pancreatic-type (pt) RNase family. Consequently, the extracellular ribonucleases expressed in tissues other than pancreas and also found in several fluids (characterized by having sequence and catalytic properties similar to those of bovine kidney RNase k2 [18] or human eosinophil-derived neurotoxin (EDN)/liver RNase [17, 19, 20]) constitute the nonpancreatic-type (npt) RNase family. Here, as already suggested [13, 17], the designations 'pt' and 'npt' have been adopted, which might be more appropriate and less confusing than the terms 'secretory' and 'nonsecretory' usually used in the past to indicate the two major classes of mammalian RNases. Other members of the RNase A superfamily (for example human plasma RNase 4 [21, 22] as well as bovine liver RNase BL 4 [23] and porcine liver RNase PL 3 [24]), being structurally more similar to mammalian ptRNases but sharing some catalytic properties [21–26] with both pt and npt ribonucleases, have been grouped indeed into a third distinct RNase family and referred to as pt/nptRNases [13]. Moreover, human angiogenin (an atypical ribonuclease distinguished by its potent angiogenic action linked to a weak unusual ribonucleolytic activity [27–29]) may constitute, together with other mammalian angiogenins, a fourth RNase family whose members could be designated as angRNases.
At present five structurally distinct proteins with RNase activity have been isolated by several laboratories from human tissues and fluids and, in addition, several variants of the same gene product have been shown to exist. Recently, the existence of a sixth human RNase has been reported [30]. But so far, this novel human ribonuclease, named RNase K6, has been identified only in the genomic DNA, and a single messenger RNA (mRNA) transcript (1.5 kb) was detected in many human tissues [30]. Thus, according to the genetic nomenclature recently introduced [22, 31] and on the basis of the designations proposed above, the six structurally defined human ribonucleases shown in figure 1 can be classified as follows.

ptRNase 1

This enzyme [16] has been isolated mostly from pancreas [15], but enzymes which are products of the same gene have also been purified from urine [32], seminal plasma [33], brain [34] and kidney [35]. In fact, only one gene coding for this RNase has been detected in human DNA by Southern blot analysis [36], and the amino acid sequence derived from the DNA sequence [37] was shown to be identical to the protein sequence [16]. In addition, the sequence analysis of a complementary DNA (cDNA) isolated from a pancreas cDNA library revealed an entire open reading frame encoding the mature protein (128 amino acids) following a 28-amino acid signal peptide [38]. However, differential posttranslational processing occurs; in this enzyme, depending on the tissue origin, the glycosylation patterns of the three Asn-Xaa-Thr/Ser sites (Asn-34, Asn-76, Asn-88) are, indeed, quite different. Each of the three sites is glycosylated in the urine enzyme, while in the pancreatic and seminal enzymes only Asn-34 was shown to have attached carbohydrates [16, 39]; more recently, using enzyme preparations obtained from fresh human pancreas, it has been demonstrated that Asn-76 and Asn-88 also had carbohydrate in about half of the molecules [40]. While no carbohydrate-free RNase was found in kidney, about half of the enzymes from seminal plasma and cerebrum are nonglycosylated [33, 34]. The primary structure of this enzyme (fig. 1) shows 70% identity with that of bovine RNase A, most of the amino acid substitutions being conservative [16, 39]. In addition, the C-terminal amino acid sequence of the enzyme purified from fresh human pancreas has been reinvestigated, and the Thr-128 was shown to be present in part of the molecules, indicating partial degradation by carboxypeptidase [40].

nptRNase 2

This enzyme [41] (also named EDN [19]) occurs predominantly in spleen [42], eosinophils [43], liver [8] and placenta [44], but has been also isolated from urine [32] and kidney [35]. All these proteins (with quite similar glycosylation patterns [45]) are products of the same

gene, which was cloned [19, 46] and located on chromosome 14 (region q24–q31) [31]. Human nptRNase 2 is synthesized as a preprotein with a 27-amino acid signal peptide [19, 31, 46]; but the true leader sequence may be shorter (23 amino acids), and the mature protein (134 residues) could be formed by signal peptidase cleavage of the Gly(-5)-Ser(-4) sequence, followed by further removal of the remaining four N-terminal residues Ser(-4), Leu(-3), (His(-2), Val(-1) [19, 31, 47]. It is worth noting that the enzymes isolated from both eosinophils [43] and liver [8] have been shown to possess a potent neurotoxicity linked to their RNase activity [20]. In the sequence of this RNase (fig. 1) five amino acid positions (corresponding to the positions 14, 62, 68, 85 and 93 of RNase A) with asparagine-linked carbohydrates have been identified [41], and their N-glycan structure [45] is very different from that reported for ptRNase 1 [40]. In addition, in the urine enzyme a novel posttranslational modification (an α-mannopyranosyl residue C-glycosydically attached to the 2 position of the indole ring of Trp-7) has recently been demonstrated [48]. The primary structure of nptRNase 2 (fig. 1) is only 35% identical to that of ptRNase 1. The major differences (RNase A numbering system) are: (i) a six-residue deletion (positions 20–25); (ii) the insertion of two, two and nine residues in three external loops of the molecule; and (iii) the addition of three residues at the amino terminus. In particular, several amino acids that are considered important in mammalian ptRNases, like Lys-7 and Arg-10 (P2 subsite), Lys-66 (Po subsite) and Phe-120 are replaced in a nonconservative way [41].

nptRNase 3

This protein [31, 49], also named eosinophil cationic protein (ECP), isolated thus far only from granulocytes [43], is very basic (pI 10.8) and highly homologous (70% identity) to nptRNase 2 [49]. Except for a deletion in nptRNase 3 corresponding to the position 116 of nptRNase 2, all changes between these two proteins (fig. 1) are due to substitutions. Along with nptRNase 2 and other human RNases, the coding sequence of this RNase maps to human chromosome 14 [31]. Human nptRNase 3 is synthesized as a preprotein of 160 amino acids, and the mature protein (133 amino acids) [31, 49] is probably formed [47] as mentioned for nptRNase 2. In the sequence of this RNase (fig. 1) there are three N-linked glycosylation sites with complex oligosaccharides, possibly containing also sialic acid [49]. Human nptRNase 3 lacks Trp-7, which is the site of the aldohexopyranosyl modification in the urine nptRNase 2. This protein is less neurotoxic than nptRNase 2, but is a more potent helmintotoxin also having antibacterial

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                  1         10        20        30        40
RNase A      KETAAAKFERQHMDSSTSAASSSNYCNQMMKSRNLTKDRCKPV
RNase 1   ---KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCKPV
RNase 2   KPPQFTWAQWFETQHINMTSQQ------CTNAMQVINNYQRRCKNQ
RNase 3   RPPQFTRAQWFAIQHISLNPPR------CTIAMRAINNYRWRCKNQ
RNase 4   ---QDGMYQRFLRQHVHPEETGG-SDRYCNLMMQRRKMTLYHCKRF
RNase 5   --QDNSRYTHFLTQHYDAKPQGR-DDRYCESIMRRRGLTSP-CKDI
RNase 6   WPKRLTKAHWFEIQHIQPSPLQ------CNRAMSGINNYTQHCKHQ

                       50        60        70        80        90
RNase A   NTFVHESLADVQAVCSQKNVACKNGQT--NCYQSYSTMSITDCRETGSS
RNase 1   NTFVHEPLVDVQNVCFQEKVTCKNGQG--NCYKSNSSMHITDCRLTNGS
RNase 2   NTFLLTTFANVVNVCGNPNMTCPSNKTRKNCHHSGSQVPLIHCNLTTPS
RNase 3   NTFLRTTFANVVNVCGNQSIRCPHNRTLNNCHRSRFRVPLLHCDLINPG
RNase 4   NTFIHEDIWNIRSICSTTNIQCKNGKM--NCHEGV--VKVTDCRDTGSS
RNase 5   NTFIHGNKRSIKAICENKNG---NPHRE-NLRISKSSFQVTTCKLHGGS
RNase 6   NTFLHDSFQNVARVCDLLSIVCKNRRH--NCHQSSKPVNMTDCRLT-SG

                            100       110                 120
RNase A   K--YPNCAYKTTQANKHIIVACEGN---------PYVPVHFDASV----
RNase 1   R--YPNCAYRTSPKERHIIVACEGS---------PYVPVHFDASVEDST
RNase 2   PQNISNCRYAQTPANMFYIVACDNRDQRRDPPQYPVVPVHLDRII----
RNase 3   AQNISNCRYADRPGRRFYVVACDNRD-PRDSPRYPVVPVHLDTTI----
RNase 4   R--APNCRYRAIASTRRVVIACEGN---------PQVPVHFDG------
RNase 5   P--WPPCQYRATAGFRNVVVACENG-----------LPVHLDQSIFRRP
RNase 6   K--YPQCRYSAAAQYKFFIVACDPPQ-KSDPP-YKLVPVHLDSIL
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Figure 1. Alignment of the amino acid sequences of the six structurally defined human RNases with that of bovine pancreatic RNase A. Identical residues in six or all seven sequences are indicated (boldface type). The references for the sequences are given in the text.

activity as well as showing cytotoxicity for mammalian cells in vitro [49]. While its neurotoxicity seems to be linked to its ribonucleolytic activity [20], RNase activity is not essential for cytotoxicity [50]. Its ribonucleolytic activity is much lower than that of nptRNase 2, but otherwise catalytic properties are similar [51].

pt/nptRNase 4

This enzyme [22] was first isolated from tumour-cell-conditioned medium [21] and later from normal human plasma [22]. The coding sequence of RNase 4 has been localized on human chromosome 14, and interestingly a large (2 kb) mRNA coding for this RNase was detected by Northern analysis in a number of human somatic tissues, including pancreas, liver, lung, skeletal muscle, heart, kidney and placenta, but not brain [52]. In addition, a cDNA sequence (996 bp) was isolated from a human pancreas cDNA library and sequenced [53]. The nucleotide sequence analysis revealed an entire open reading frame encoding the mature protein (119 amino acids) following a 28-amino acid signal peptide [53]. No N-linked glycosylation sites have been observed in the sequence of pt/nptRNase 4, the only posttranslational modification being the pyroglutamic acid found at its amino terminus [22]. This protein has a primary structure (fig. 1) more like that of human pt RNase 1 (43% identity) than those of nptRNase 2 and nptRNase 3 (31% and 30% identity, respectively), but shows the same substrate preference of nptRNases; it strongly prefers poly(U) over poly(C) showing indeed an activity ratio much higher than those of npt RNases 2 and 3 [17, 21, 22, 51]. In addition, this human RNase was shown to be highly homologous (about 90% identity) to porcine and bovine liver RNases also showing a unique deletion of two residues (positions 77 and 78 of RNase 1) [22–24].

angRNase 5

This protein [54] (called angiogenin), originally isolated from tumour-cell-conditioned medium on the basis of its potent in vivo angiogenic activity, was also purified from normal human plasma [55]. The sequence of the cDNA and gene for angRNase 5 was determined [56], and nucleotide sequence analysis revealed a sequence coding for a signal peptide of 24 amino acids followed by 369 nucleotides coding for the mature protein of 123 amino acids. Its principal biological role is to induce the formation of new blood vessels. Despite its remarkable structural similarity (fig. 1) to pt/nptRNase 4, ptRNase 1 and nptRNase 2 (39%, 35%, and 27% sequence identity, respectively), angRNase 5 shows an unusual ribonucleolytic activity [27, 28], which differs markedly both in magnitude and specificity from RNase activity of the other human RNases. The extremely weak ribonucleolytic activity (towards standard RNase substrates [27]) of angRNase 5 which is, however, essential for angiogenicity, seems to be in part due to the obstruction of the pyrimidine binding site (as observed in the homologous RNase A structure) by Gln-117 [29]. Recently, the structural, catalytic and biological characteristics of this protein have been reviewed in detail by J. F. Riordan [57].

nptRNase 6

This RNase [30] (also named RNase K6 for its orthologous relationship with bovine RNase K2 [18]) is the most recently discovered human member of the mammalian RNase gene superfamily. As mentioned already, this novel RNase as a protein has not been purified from any human tissue or fluid, but a genomic fragment encoding RNase K6 has been identified in human DNA and localized on chromosome 14; the open reading frame encodes a 23-amino acid hydrophobic leader sequence followed by a polypeptide of 127 amino acids. In addition, a single mRNA transcript (1.5 kb) has been detected in many tissues including heart, brain, placenta, liver, skeletal muscle, kidney and pancreas, with lung representing the most abundant source. The detection of mRNA encoding this protein in both neutrophils and monocytes suggests a role for this RNase in human host defence [30]. The deduced amino acid sequence of nptRNase 6 (fig. 1) is 47 and 72% identical [30] to the sequences of human nptRNase 2 [31, 41] and its bovine RNase K2 orthologue [18], respectively; two potential sites for asparagine-linked glycosylation are also present [30]. The enzymic activity of the recombinant protein has been tested against yeast RNA only, and a kinetic comparison with recombinant human RNase 2 indicates that nptRNase 6 is about 40-fold less active than nptRNase 2 [30].

Catalytic features and other biochemical properties

The six structurally defined human RNases described above, despite their remarkable sequence similarities (fig. 1) are catalytically quite different. Excluding angRNase 5 because of its unusual ribonucleolytic activity, as well as nptRNase 6, for which no data are available thus far, the remaining four RNases show catalytic properties which are characteristic of one or both the two major RNase types (ptRNase 1 and nptRNase 2); these properties will be comparatively analysed and discussed in the following sections.

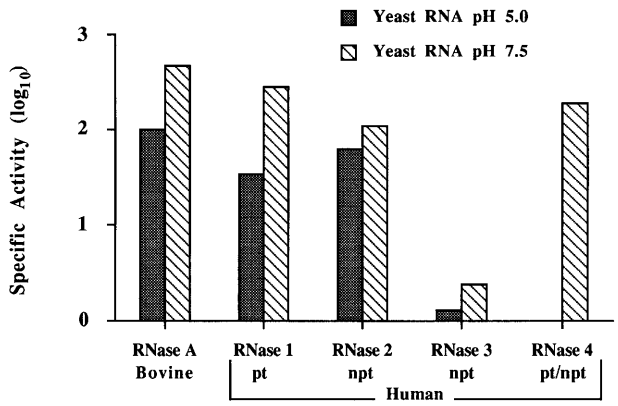

Figure 2. Activity of human RNases and bovine RNase A towards yeast RNA. Specific activity, Kunitz units/mg of protein. Figure prepared with data published in refs 17, 33, 51, 20, 21. The activity of pt/npt RNase 4 towards yeast RNA has not been measured [21] at pH 5.0.

Activity toward yeast RNA and influence of ionic strength and cations

Human RNases (except nptRNase 3 and nptRNase 6) degrade yeast RNA (fig. 2) with activity values comparable to those of bovine RNase A (i.e. 2.5–4 times lower, depending on the enzyme and experimental conditions). Towards this substrate, nptRNase 3 and nptRNase 6 are indeed two orders of magnitude less active than the bovine enzyme [30, 51, 58]. The activity of human ptRNase 1 and nptRNase 2 towards yeast RNA has been shown to be differently influenced by ionic strength and divalent cations [17]. The activity of ptRNase 1 is enhanced by an increase of the NaCl concentration from 50 to 300 mM, while the activity of nptRNase 2 decreases above 150 mM. Another difference between the two RNase types is that while the activity on yeast RNA of ptRNase 1 is strongly inhibited by low zinc ion concentrations (0.5 mM), that of nptRNase 2 does not seem to be significantly influenced. Magnesium ions, instead, have no effect on the activity of either RNase type [17]. This could be partly ascribed to a stoichiometric bond possibly forming in ptRNase 1 between a zinc ion and His-12 and His-119, but not in nptRNase 2 [17] because of structural differences of its catalytic site [59].

Substrate preference

As summarized in figure 3, human ptRNase 1 (like other mammalian ptRNases) is about 30-fold more effective towards poly(C) than poly(U) [17]. On the contrary, nptRNase 2 and nptRNase 3 as well as pt/nptRNase 4 prefer poly(U) over poly(C); in particular, while nptRNases 2 and 3 show a poly(U)/poly(C) activity ratio of 4 and 12, respectively [17, 51], pt/nptRNase 4 degrades poly(U) at least 1000-fold faster than poly(C) [21, 22]. This preference [17, 21, 22, 32, 35, 42, 51] seems to be due to a significant reduction of their affinity for poly(C), rather than to an increased preference for poly(U) and could be attributed to the different microenvironment of the B1 subsite of both nptRNases and pt/nptRNase 4. In nptRNases 2 and 3 (fig. 1) in fact, Thr-45 (RNase A numbering) is conserved but Ser-123 is not, and this might prevent productive binding of the cytosine moiety of the substrate molecule. Thr-45 is conserved also in human pt/nptRNase 4, and the truncation of the sequence at position 122 (RNase A numbering) was suggested [22] to be responsible for the dramatic loss in activity of this RNase towards cytidine dinucleotides. However, Ser-123 is replaced by other residues (Thr, Tyr) in several mammalian ptRNases [10]. Recently, it has been suggested [59] that in nptRNase 2 thq side chain of Gln-40 (Val-43 in RNase A) can either accept or donate hydrogen bonds, like Ser-123 of RNase A, and could be responsible for binding the O-4 of uracil or N-4 of cytosine. It is worth noting here that, like nptRNase 2, nptRNase 6 (fig. 1) also lacks Ser-123 but has Gln-40 (position 43 of RNase A). At present no catalytic information on this point concerning RNase 6 is available, but a substrate preference similar to that of npt RNase 2 should be expected.

Human pt/npt RNase 4 contains a phenylalanine residue at position 42 (Val-43 in RNase A) which has

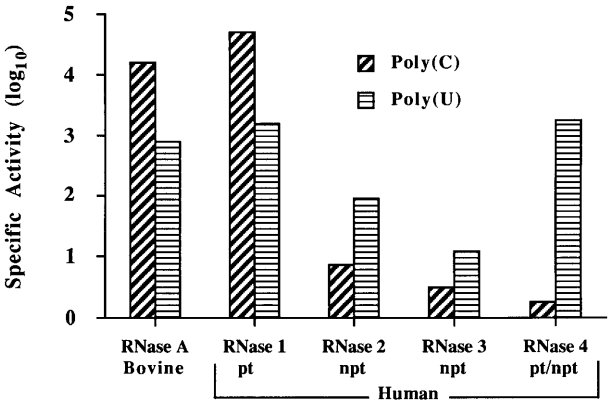

Figure 3. Substrate preference of human ribonucleases compared with that of bovine pancreatic RNase A. Specific activity, units/mg of protein; units defined as change in absorbance at 260 nm/min/total mesurable change. Figure prepared with data published in refs 17, 21, 51. The data concerning pt/nptRNase 4 (obtained by Shapiro et al. [21]) were transformed into the enzyme units defined in [17, 51] and included in the figure only to allow a rough comparison.

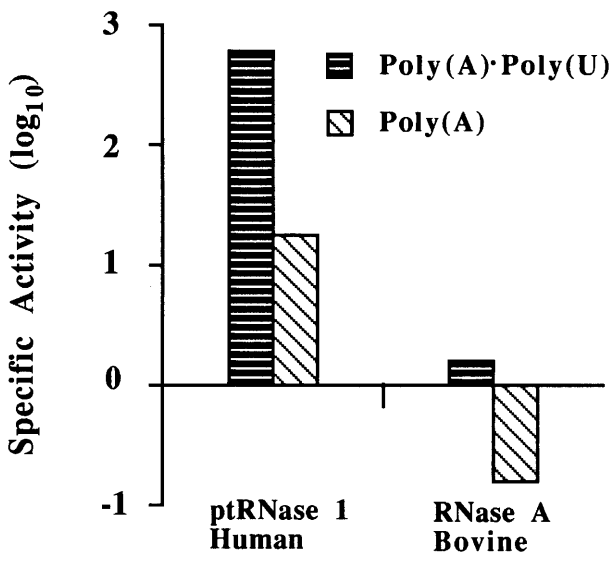

Figure 4. Action of human ptRNase 1 and bovine pancreatic RNase A towards poly(A) and double-stranded polyribonucleotides. Specific activity, as defined in the legend of Figure 3. Figure prepared with data published in ref. 17.

been suggested to play an important role through its possible interaction with uracil [22]. The porcine homologue RNase PL 3 also strongly prefers poly(U) over poly(C), and it was proposed [25] that the 36–42 region of this enzyme is involved in discriminating between uridine and cytidine. Recently [26], site-directed mutagenesis of amino acids in this region demonstrated that Phe-42 is the only single residue which affects the poly(U) preference of this RNase type, partially through an effect on Thr-44 (Thr-45 in RNase A).

Activity on double-stranded polyribonucleotides and poly(A)

Another notable difference between the two major human RNase types is that ptRNase 1 towards poly(A) and double-stranded poly(A) · poly(U) is about two orders of magnitude more active than RNase A (fig. 4), whereas nptRNases 2 and 3 are totally inactive on those substrates [17, 35, 42, 51]. Recently, it was suggested that in bovine RNase A [60] (and very probably in all mammalian ptRNases) Asp-83 could play an important role in activity towards poly(A). Interestingly, nptRNases [inactive on poly(A)] at the corresponding position lack Asp-83. But in pt/npt RNase 4 (inactive on both poly(A) and poly(G) [21]) this residue has been conserved (fig. 1).

The enzymatic cleavage of double-stranded RNA (dsRNA) by ptRNases may occur, according to a mechanism already proposed [17, 61], as the consequence of the preferential binding of the RNase molecule to short single-stranded sequences of the substrate transiently exposed by spontaneous thermal fluctuations. This model is supported by the observation that human ptRNase 1 and other mammalian ptRNases show a remarkable DNA-helix-destabilizing action, while nptRNase 2 as well as nptRNase 3 have no such activity [17, 51, 61]. A complementary model, based on the binding of the enzyme to single nucleotides wound off the double-helix, was also advanced [62].

Bovine RNase A has a DNA-unwinding activity (lower than that shown by human ptRNase 1 [61]) which has been related to the multiplicity of phosphate-binding subsites (Lys-7, Lys-41, Lys-66, Arg-85, Arg-39, Lys-91 Lys-98, Arg-33, Lys-31) of the enzyme protein [63]. In this respect, the higher double-helix-destabilizing action and dsRNA-degrading activity shown by human ptRNase 1 [17] could be explained by a stronger local positive electrostatic potential developing on the human enzyme because of the presence of basic amino acid residues (Arg-4, Lys-6, Arg-32, Lys-102) at positions where in bovine RNase A neutral residues are present instead [14, 16]. It is worth noting here that additional positive charges have been also found in the sequences of other mammalian ptRNases characterized by having a remarkable activity towards dsRNA [61]. In particular, in the sequences of these ribonucleases [9], basic residues have been found at the following positions: position 6 (pig RNase), position 32 (pig, horse and whale RNases), position 34 (whale and bovine 'seminal' RNases) and position 102 (horse and whale RNases). Another basic residue possibly involved in determining a remarkable activity on dsRNA may be Arg-39; in fact, in RNase A (low activity on dsRNA) the possible interaction between Arg-39 and the phosphates of the dsRNA substrate may be disturbed by the presence of the side-chain negative charge of Asp-38. Human ptRNase 1, instead, as well as pig, whale and bovine 'seminal' RNases (about 20-fold more active than RNase A towards dsRNA [61]) lack Asp-38, showing at this position a glycine residue [9]. Interestingly, human pt/npt RNase 4 lacks Arg-39, and although it has two additional positive charges at positions 32 and 34 (in comparison with RNase A), it has recently been shown to degrade poly(A) · poly(U) with a specific activity much lower than that of human ptRNase 1 and more like that of RNase A (S. Sorrentino, unpublished results). Another significant observation is that human nptRNases 2 and 3, although higly basic proteins, do not show the extended multisite cationic region which characterizes mammalian

ptRNases [63] and, as mentioned, are unable either to destabilize the DNA secondary structure or to degrade dsRNA [17, 51]. These arguments seem to support the idea already advanced [17, 61] that both helix-destabilizing action and degrading activity shown by a mammalian ptRNase may depend on the number and cooperativity of electrostatic interactions between the phosphates of the substrate and 'specifically located' positive groups of the RNase molecule. Recently, the kinetic parameters of enzymic degradation of single- and double-stranded polyribonucleotides by the action of some human and bovine ptRNases have been studied comparatively and discussed in the light of this model [64].

In conclusion, on the basis of the observations mentioned above, while mammalian ptRNases might by now be classified as single-strand/pyrimidine 'preferring' ribonucleases, human nptRNases (and possibly also other mammalian nptRNases) may be defined as true single-strand/pyrimidine 'specific' ribonucleases.

pH-dependence of RNase activity and hydrolysis of cyclic nucleotides

Human ptRNase 1 and nptRNase 2 differ in two other important points: their pH optima with RNA as a substrate, and the so-called 'second step' of the ribonuclease reaction [17]. The pH optimum for the activity of human ptRNase 1 towards RNA is 8.0, and the enzyme efficiency at hydrolysing 2′,3′-cyclic nucleotides is comparable with that of bovine RNase A [15, 17, 32, 34, 35]. The pH optimum for the degradation of yeast RNA by nptRNase 2 is instead between 6.5 and 7.0 [17, 32, 35, 42], whereas the ability of the enzyme to hydrolyse cyclic nucleotides is practically nil [17, 20, 32]. The same inability characterizes nptRNase 3 [51]. For a possible explanation of these differences, the amino acid residues (RNase A numbering system) specified in table 1 are particularly important.

The importance of Lys-66 has been recognized for all mammalian ptRNases [63, 65]. In addition, Beintema has suggested that a basic amino acid residue located at either position 66 or 122 could be of great importance to 'normal' ribonucleolytic activity [65]. It is quite interesting to note (table 1) that human nptRNase 2 and turtle RNase are characterized by the absence of Lys-66 but also by the presence of a basic amino acid residue at position 122; accordingly, they show 'good' RNase activity [10, 65]. Angiogenin, nptRNase 3 and onconase (an RNase from *Rana pipiens* oocytes) [66], in which a basic amino acid is absent at both positions 66 and 122, do indeed show a very modest ribonuclease activity [8, 51, 65, 66].

Asp-121 plays an important role in the catalytic mechanism of bovine RNase A, as shown by site-directed mutagenesis experiments [67]. In RNase A the existence of a possible interaction between Asp-121 and His-119 has been proposed [68]; this interaction, at pH 8, could be of a particular importance in the stabilization of the positive charge on His-119. Asp-121 is a conserved residue in human nptRNase 2 (fig. 1) and also in bovine kidney (npt) RNase K2 [18, 69] as well as in turtle RNase [10, 65]. These enzymes, however, have a basic amino acid at position 122 (where ptRNases show a neutral residue) which could play a crucial phosphate-binding role [65], though it may influence the possible interaction between Asp-121 and His-119 and therefore be responsible for a lower pH value (6.5–7.0) necessary to maintain His-119 in its protonated form. The lower pH optima of human ptRNase 2 and bovine RNase K2 (table 1) support this hypothesis. Unfortunately, no information concerning the optimal pH of turtle RNase or human nptRNase 6 is presently available. However, on the basis of the arguments advanced above, for turtle RNase (even though it is a pancreatic enzyme) an optimal pH similar to that of nptRNases should be expected, while human nptRNase 6 (which at position 122 shows a polar residue) might have a pH optimum slightly lower than that of ptRNases. Onconase (table 1) at position 122 has a neutral residue but lacks Asp-121. The observed value of its pH optimum is in accordance with the hypothesis advanced above: when the

Table 1. Structural and catalytic characteristics of human RNases compared with those of other members of RNase superfamily.

Enzyme	Position 66	Phe-120	Asp-121	Position 122	pH optimum on yeast RNA	Activity on cyclic nucleotides
Bovine RNase A	Lys	+	+	Ala	8.0	+
ptRNase 1	Lys	+	+	Ala	8.0	+
pt/nptRNase 4	Lys	+	+	Ala	7.5	+
Human RNase K6	Lys	−	+	Ser	?	?
Bovine RNase K2	Lys	−	+	Lys	6.5	?
nptRNase 2	Pro	−	+	Arg	6.5–7.0	−
Turtle RNase	Ser	−	+	Lys	?	−
Onconase	deletion	+	−	Gly	6.0	+

His-119/Asp-121 interaction is disturbed or is absent, a pH value lower than 8 seems to be necessary to maintain the positive charge on His-119.

As mentioned already, although human nptRNase 2 is capable of efficiently degrading RNA, it does not show any detectable hydrolytic activity towards 2′,3′-cyclic phosphodiesters [17, 20, 32] which have been considered for many years to be intermediates in the RNase A-catalysed degradation of RNA. Regarding this point, it has been demonstrated with several ptRNases that the 2′,3′-cyclic phosphodiesters are not enzyme-bound intermediates but are true reaction products which are released into the solution and hydrolysed in a separate slower reaction [63, 70, 71]. From table 1 it appears that the ribonucleases active on cyclic nucleotides present a phenylalanine at position 120, while nptRNase 2 and turtle RNase do not show any detectable activity on the same substrates and lack Phe-120. Interestingly, human RNase K6 and bovine RNase K2 lack Phe-120, but no experimental data concerning these observations are available. However, some experiments performed with bovine RNase A and human angiogenin [72, 74] seem to indicate that the absence in an RNase molecule of the aromatic side-chain of Phe-120 does not influence the transphosphorylation reaction, but it may be responsible for the inability of the enzyme to efficiently catalyse the hydrolytic reaction of cyclic phosphodiesters. In conclusion, the observations discussed above may support the suggestion already advanced [70, 71] that the enzyme 'ribonuclease', presently classified as 'hydrolase' should be reclassified as 'transferase'.

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