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Hypothesis

Presence of a basic amino acid residue at either position 66 or 122 is a condition for enzymic activity in the ribonuclease superfamily

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Some members of the ribonuclease superfamily differ at more than 50% of the amino acid positions. Although the three-dimensional structures probably are very similar and the active-site residues have been conserved, other substrate-binding regions have changed considerably. Several proteins in the superfamily are active ribonucleases while others exhibit practically no enzymic activity. The presence of a basic residue at either position 66 or 122 appears to be a condition for ribonuclease activity.

Ribonuclease superfamily; Amino acid position; Active site conservation; Ribonuclease activity

Pancreatic ribonucleases (EC 3.1.27.5) are enzymes which cleave RNA endonucleolytically to yield 3′-phosphomono- and -oligonucleotides ending in Cp or Up, with 2′,3′-cyclic phosphate intermediates. The enzyme from bovine pancreas is one of the most intensively studied proteins and much information has been collected about its structure and functional properties [1]. The residues in the active site that take care of cleavage of the phosphodiester bonds in RNA are well characterized, and there are indications of several surface positions where other parts of the macromolecular substrate may bind [2–4]. Although pancreatic ribonucleases from other mammalian species [5] and other so-called secretory ribonucleases, like bovine seminal [6] and brain [7] ribonuclease as well, may differ in as many as one-third of the amino acid residues in the sequence, the general structural organization of the enzyme has not changed and residues considered to be important for catalysis and substrate binding have been conserved [5].

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Recently the amino acid sequences of a number of more distantly related members of the ribonuclease superfamily have been determined. These proteins differ at more than 50% of the amino acid positions from mammalian pancreatic ribonucleases, and several gaps/insertions have to be introduced to attain proper alignment. These proteins are listed in table 1, and fig.1 presents the aligned amino acid sequences of four of them. The majority of these proteins have ribonucleolytic activity and can be classified as ribonucleases, but the two angiogenins and human eosinophil cationic protein have a very low activity on RNA.

All proteins listed in table 1 probably have about the same three-dimensional structure as bovine pancreatic ribonuclease. All insertions and gaps can be accommodated at external bends [11,17]. In addition, a number of amino acid residues are conserved. These include the active-site residues His-12, His-119, and Lys-41, 6 half-cystine residues which form three of the four disulfide bonds in bovine ribonuclease, and 9 other residues

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Table 1

Members of the ribonuclease superfamily

	Ribonuclease activity[a]	References
Mammalian pancreatic ribonucleases	+	1,5
Bovine seminal ribonuclease	+	6
Bovine brain ribonuclease	+	7
Turtle pancreatic ribonuclease	+	8
Human angiogenin	−	9
Bovine angiogenin	−	10
Human nonsecretory ribonuclease/ eosinophil-derived neurotoxin	+	11,12,13, 20, 21
Human eosinophil cationic protein	−	12,13, 22
Bovine nonsecretory ribonuclease	+	14
Frog liver ribonuclease	+	5
Frog egg lectin	+	5,15

[a] Ribonuclease activity: +, active ribonuclease; −, less than 5% of the specific activity of bovine pancreatic ribonuclease

(table 2). Although the three active-site residues most directly involved in catalysis have been conserved, other residues considered to be important for enzymic activity or substrate binding have been replaced. The very low ribonuclease activities of angiogenin and the eosinophil cationic protein could be explained if only certain amino acid residues were to be replaced in these latter proteins. However, there are no such residues as will be shown in the following examples.

The aromatic residue (Phe or Tyr) at position 120 in mammalian ribonucleases is in direct contact with the pyrimidine ring of the substrate in the primary specificity site of the enzyme [1,2]. This residue is replaced in human but not in bovine angiogenin [9,10]. In studies on semisynthetic derivatives of bovine ribonuclease, the replacement of this residue by leucine has been found to lower the specific activity about tenfold [18].

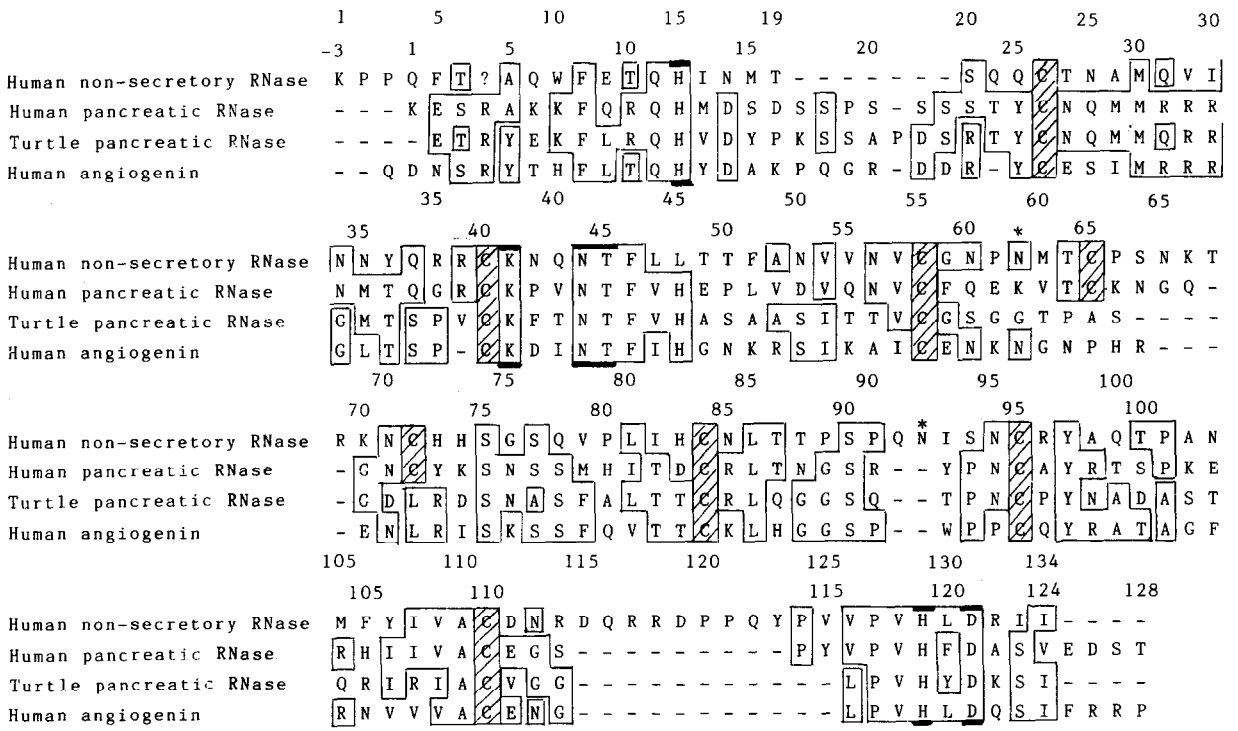

Fig.1. Comparison of the amino acid sequences of human nonsecretory ribonuclease [11], human pancreatic ribonuclease [16], turtle pancreatic ribonuclease [8], and human angiogenin [9]. Positions are numbered according to the residue number of human nonsecretory ribonuclease (upper line) or human pancreatic ribonuclease (lower line). (−) No amino acid residue present; (?) identity unknown. Identical residues in two or more sequences are enclosed in blocks. (Hatched lines) Half-cystine residues; (heavy lines) residues in the active site or involved in substrate binding in bovine pancreatic ribonuclease.

Table 2

Invariant residues in members of the ribonuclease superfamily

Phe-8	Asn-44	Cys-95
His-12	Thr-45	Tyr-97
Cys-26	Phe-46	Cys-110
Met-30	Cys-58	Pro-117
Cys-40	Ser-75	Val-118
Lys-41	Cys-84	His-119

Harper et al. [19] studied the enzyme properties of a complex of bovine pancreatic ribonuclease (residues 1–118) and a peptide with the amino acid sequence of the C-terminal 16 residues of human angiogenin. A low ribonuclease activity was found. Replacement of leucine at position 120 by phenylalanine increased the activity toward low-molecular mass substrates up to 100-fold, but hardly influenced the activity toward RNA. The presence of leucine at position 120 in human non-secretory ribonuclease also indicates that this replacement cannot explain the low ribonuclease activity of human angiogenin.

Another region which may have a role in the enzymic activity is the surface loop with the disulfide bond connecting residues 65 and 72 in bovine ribonuclease. It has several substrate-binding sites. This loop is shorter in turtle ribonuclease and angiogenin, and the two half-cystine residues which form a disulfide bond have also been replaced in these proteins. There is an insertion in this loop in human non-secretory ribonuclease. If this region is important for substrate binding in bovine pancreatic ribonuclease, there should be other secondary binding sites for substrate in the proteins with ribonuclease activity that have changed extensively in this loop region. One of the more important interactions is that between the side chain of lysine 66 and the 5′-phosphate group of the nucleotide in the primary pyrimidine-binding site. This phosphate-binding site is called the P_o site [4]. Lysine 66 is present in all mammalian pancreatic and other secretory

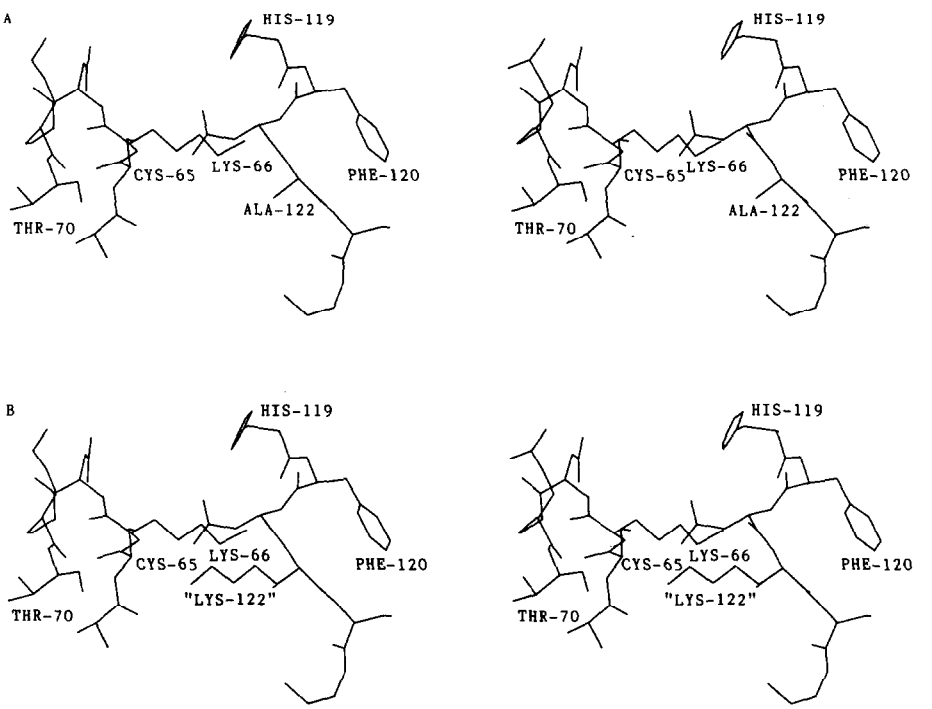

Fig.2. (A) Stereo drawing of part of the structure of bovine pancreatic ribonuclease including active-site residue His-119 and the substrate-binding residues Tyr-120 and Lys-66. (B) Same figure with a lysine side chain at the position of Ala-122, demonstrating the close vicinity of its positively charged end group and that of Lys-66.

ribonucleases [5], but has been replaced in members of the ribonuclease superfamily that have deletions or insertions in this region. However, turtle pancreatic ribonuclease and human non-secretory ribonuclease have lysine and arginine, respectively, at position 122 (fig.1). The positive charge of this residue at position 122 probably can take over the role of lysine 66 in pancreatic ribonucleases (fig.2). Human and bovine angiogenin and human eosinophil cationic protein have no basic residue at either position (fig.1; [9,10,22]. This may explain the very low ribonuclease activity of the latter proteins.

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