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Pancreatic Ribonuclease

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Gene Symbol: RNASE1

1. General

Pancreatic ribonuclease also known as ribonuclease A (RNase A) or ribonuclease 1 (RNase1) is an enzyme that catalyzes the breakdown of RNA and plays a role in the digestion of RNA in vertebrate species. Early work focused on bovine pancreatic RNase because of the large amount present in the pancreas. It has been described as the best studied enzyme of the 20th century with four Nobel prizes awarded for studies of this protein (20). Owing to the high levels present in the bovine pancreas, RNase1 was historically considered as a digestive enzyme but with little purpose in humans and other non-ruminant mammals (2) where it exists at much lower concentrations. However, digestion of RNA occurs in the intestine of all species and RNase1 and other members of its superfamily are now known to have additional functions in host defense that will be discussed later.

Structure and Mechanism of Action

Bovine RNase1 was purified and crystalized by Kunitz in 1939 (16) and sequenced by Smyth, Stein and Moore in 1963 (30). It contains 124 amino acids, a calculated molecular weight of 13,683 and an excess of basic residues leading to an isoelectric point of 8.5 – 9.0. It contains four disulfide bonds and has been used as a model to study protein folding (22). It is also glycosylated on asparagine residues with the glycosylated form originally termed RNase B. Because of the glycosylation and tertiary structure, it runs on most SDS gels at 18 or 19 kDa. Porcine RNase 1 contains 125 amino acids (26); human RNase 1 contains 3 additional amino acids at the carboxyl terminal than bovine RNase1 (4). Bovine RNase1 was the first enzyme to have its three-dimensional structure determined. It has an overall shape like a kidney bean with the active site in the cleft containing His12, His119, and Lys41 (25). Other non-catalytic binding sites help the enzyme to form a complex with its polymeric substrate. RNase1 catalyzes the hydrolysis of 3',5'-phosphodiester linkages in single stranded RNA when the base on the 3' side is a pyrimidine (7, 11, 23). The process is usually represented as occurring in two stages with the first step involving the formation of a cyclic phosphodiester and the second it's hydrolysis (23). RNase 1 does not hydrolyze DNA as it lacks a 2'-OH group. This allows it to be used to remove RNA contamination from DNA. It is also used in ribonuclease protection assays. RNase 1 forms dimers by domain swapping of amino termini by sulfhydryl bond formation in such a way as to keep each active site active (18). While retaining

hydrolytic function the dimers acquire an additional biological activity with dimers, trimers and tetramers possessing anti-tumor activity.

Ribonuclease A Superfamily

The human genome codes for 122 separate ribonucleases. The RNAse A superfamily consists of eight “canonical” ribonucleases with enzymatic activity and structural homology to RNase A (15). All are secretory proteins that share a disulfide bonded tertiary structure and are able to degrade RNA. All are coded for in a tight region of chromosome 14 in both humans and mice (27) and are believed to have originated from gene duplication (3, 31). Though the gene contains several exons, the coding region is contributed by a single exon. Understanding of their physiological role is incomplete but most are important for host defense and angiogenesis as well as digestion (9). The first member to be described is RNase 1 which in addition to being a pancreatic enzyme is produced in a variety of cells including vascular endothelial cells where after secretion it degrades vascular polymeric RNA and has anti-HIV-1 activity (15). RNase 2 and 3 are eosinophil secretory proteins termed eosinophil derived neurotoxin (EDN) and eosinophil cationic protein (ECP) respectively (15). RNase 4 is present in multiple tissues but it’s physiologic role is unclear. RNase 5, also known as angiogenin, induces blood vessel growth (10). RNase 7 is the most abundant RNase in skin while RNase 8 is expressed in the placenta (15). Additional genes in the cluster are related to ribonucleases (RNase 9 to 13) but their proteins have mutations preventing RNase activity. Some of the secreted RNases or their oligomers can enter cells and exert cytotoxic effects especially on tumor cells.

Ribonuclease Inhibitor

Mammalian ribonuclease inhibitor (RI) is a cytosolic protein of 50 KDa that binds with high affinity and 1:1 stoichiometry to pancreatic ribonuclease thereby inactivating it (8, 17). It is particularly abundant in placenta and liver and has been used to purify RNase. It inhibits all members of the RNase A family. Its three-dimensional structure is that of a horseshoe which contains leucine rich repeats. Although the biological role is not clear it should bind and inactivate any RNase A family member escaping the secretory pathway and entering the cytoplasm.

2. Role of ribonuclease in the pancreas

Most cells contain millimolar concentrations of ribonucleotides but only micromolar concentrations of deoxyribonucleotides (5). Thus the diet contains a mixture of ribonucleoprotein, RNA and ribonucleotides. Nucleoproteins are broken down by pancreatic protease. The textbook view is that dietary nucleic acids are broken down by pancreatic RNase and DNase in the intestine. A recent study, however, has shown that pepsin in the stomach also hydrolyzes nucleic acid, so this digestion starts there (19). Foods rich in ribonucleoproteins include organ meats, seafood and legumes (5).

Pancreatic RNase (RNase 1) is present in all vertebrate pancreas but the amount varies greatly (2). Mammals with large amounts include ungulates, rodents and herbivorous marsupials. In the cow, RNase makes up 20% of digestive enzyme; this requirement is thought due to the large RNA load that is produced by bacteria from ruminal fermentation. In other species including humans, dog, cat and lower vertebrates, RNase is present at much lower amounts and may account for only 0.5 to 1% of pancreatic enzymes. Although only few studies exist, pancreatic RNase in all species appears to break down dietary nucleic acid in the gut lumen to nucleotides which are further broken down by intestinal alkaline phosphatase and 5’nucleotidase to nucleosides and free nitrogenous bases. Therefore, digestion of nucleic acids has a luminal and a brush border phase. These products are absorbed by enterocytes but

most are excreted in urine; some are used for resynthesis primarily in the fasting state (13, 21, 29). Normally 80 to 90% of nucleotides are absorbed and these can become essential in certain diseases or periods of limited intake or rapid growth (5).

Ribonuclease is synthesized in acinar cells by rough ER, folded and packaged into zymogen granules beginning at 20 days gestation in the rat (33). Folding occurs much more rapidly in cells compared to the isolated protein and except for a small amount of dimer any protein not ending as a folded monomer is degraded (12). It is secreted into the medium parallel to other digestive enzymes by pancreatic lobules and acini stimulated with CCK or cholinergic analogues (14, 24, 28). RNase 1 synthesis is reduced in experimental diabetes by 50% but much less than the decrease for amylase.

3. Tools for the study of Ribonuclease1

a. Antibodies

Biocompare (www.biocompare.com) lists 394 ribonuclease antibodies from 32 suppliers. Some are species specific while others are specific for RNase1 or other family members. We have not tested any of these antibodies.

b. Ribonuclease activity

Early ribonuclease assays used the hydrolysis of yeast RNA; we used an assay described by Anfinsen (1) to measure the secretion of pancreatic ribonuclease by isolated rat pancreatic acini (24). These assays however are not specific for RNase1 and worked for the study of pancreatic acinar secretion because RNase1 is the major form present in pancreatic acini. An assay has also been described using the hydrolysis of cytidine 2'-3'-phosphate (6). Recently a quantitative fluorescence assay was developed based on the binding of ethidium bromide to yeast RNA (32).

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