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Pancreatic DNase

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Gene Symbol: DNASE1

1. General Information

Pancreatic DNase (DNase 1) is one of four known mammalian DNases and is the secretory enzyme found in pancreas and parotid gland and their secreted fluid. It is also present in, seminal fluid, serum and urine with much lower amounts in some other tissues. Some consider the DNase 1 in other tissues to be distinct related enzymes that belong to the DNase 1 family with similar characteristics outlined below (13, 33). The DNase in serum and urine is believed to be of pancreatic origin. While it is considered primarily to be a digestive enzyme that functions to break down DNA in the alimentary tract (15, 32, 38) other functions have been suggested including degradation of DNA in apoptosis (45). Using native PAGE zymography to detect tissue activity and confirmed by its absence in DNase $1^{-/-}$ mice, Napirei et al. identified DNase1 in a number of tissues (27).

DNase activity in the pancreas was first identified by Sachs in 1906 (29). Following the isolation of trypsinogen and chymotrypsinogen as specific proteins, DNase 1 was isolated in crystalline form from bovine pancreas and shown to be a heat sensitive protein that could cleave thymus DNA (16). It was then shown to also be present in bovine pancreatic juice where it made up 2.4% of protein (12). It was subsequently shown to be present in multiple upper GI organs including pancreas, parotid, submandibular gland, stomach (chief cells), small intestine (Paneth Cells), as well as human duodenal juice, serum and urine (7,19, 27,32,38) When evaluated by EM immunocytochemistry using antibody raised against purified pancreatic proteins, pancreatic DNase was concentrated in the secretory pathway similar to other pancreatic secretory proteins with the highest amount in the Golgi and zymogen granules (2,14). Subsequently DNase 1 has been isolated from many mammalian species as well as chicken, snake and xenopus and it has been cloned and sequenced from 36 species. It is made up of a short N-terminal signal peptide and the mature enzyme, which contains 259 – 262 amino acids (260 for human) and, in most cases, a small amount of carbohydrate (6,11,24,42). The predicted amino acid sequence yields a protein mass of 30,200 Da, but on polyacrylamide gel electrophoresis or size exclusion chromatography it has an apparent molecular mass of 32-38 kDa and by isoelectric focusing a PI of about 3.5 – 4.0 depending on species

and technique. The structure of the human and mouse gene contains eight coding exons and is located on chromosome 16 (28,41).

Functionally, DNase 1 (EC 3.1.21.1) is a divalent cation dependent endonuclease that cleaves double stranded DNA usually on one strand and results in oligonucleotides with a 5' phospho and 3' hydroxy terminal. The cleavage is not sequence or base specific, but the rate of cutting is sequence specific (34). It is most active at pH 7.5 and can be inhibited by globular actin in most species except for rat (17,21). Monomeric actin binds in a one-to-one complex that inhibits DNase activity and the ability of globular actin to polymerize. DNase 1 binds to about 10 base pairs across the DNA minor groove. The three-dimensional structure of bovine pancreatic DNase has been determined and it consists of two 6 stranded beta sheets forming a sandwich structure (35).

DNase 1 is known to be a glycoprotein and most mammalian species evaluated have two consensus sites for N-glycosylation at Asn-18 and Asn-106 (6). The carbohydrate moieties differ among species but allow purification by lectin chromatography. Using peptide N-glucosidase F to remove the sugar chains, the enzymatic activity of human DNase 1 was shown to be decreased and the enzyme was more susceptible to heat (4). Site directed mutagenesis has shown the importance of both glycosylation sites to maximum enzymatic activity, heat stability and resistance to trypsin digestion (5). Thus, glycosylation appears important for the function of DNase 1.

There are multiple isoforms of DNase 1 whose origin and function are incompletely understood. Takeshita et al. purified DNase 1 from 14 different tissues of humans and multiple animal species. Humans and pigs had the highest concentration in the pancreas, while rat and mouse were highest in the parotid gland, and bovine and rabbit were mixed (36). They classified DNase as belonging to three types, pancreas, parotid and mixed. Pancreatic DNase 1 was shown to be acid sensitive while parotid was not. Others have shown that human and bovine DNase 1 could be separated into 4 – 8 bands on isoelectric focusing (37). Abe and Liao (1) reported that the difference between bovine pancreatic and parotid DNase was due to their sugar chains and not the protein sequence. However, Yasuda et al. reported two individuals exhibiting genetic polymorphism that differed in one base producing either Gln or Arg at position 222 of the mature enzyme (41). Most authors over the last decade considered that differences in both carbohydrate and protein contribute to the multiple isoforms although the functional significance of these isoforms is not clear.

2. Pancreatic DNase and Digestion

Nucleic acids enter the GI tract as part of both animal and plant food, as well as in sloughed mucosal cells lining the gut. DNA is a linear polymer of nucleotides containing deoxyribose linked together by phosphodiester bonds. The precursors for DNA synthesis are dATP, dGTP, dCTP, and dTTP. These nucleotides can be synthesized in the body, so they are not essential nutrients. In addition to being used to make new nucleic acids, dietary nucleotides may have beneficial effects on intestinal growth, the immune system and hepatic metabolism (3).

Acid in the stomach separates proteins such as histones from DNA which is primarily in the form of a double helix. The DNA is broken down into small fragments termed oligonucleotides initially by the gastric enzyme pepsin (18) and then to a greater extent by pancreatic DNase 1. Pepsin uses the same active site which breaks

down peptide bonds to cleave DNA. The importance of pancreatic DNase in digestion may be questioned as mice with a genetic deletion of DNase 1 grow normally (26). However, actual digestion of DNA was not measured in these animals and DNA can be synthesized de novo.

Small amounts of nucleotides are absorbed by the small intestine, but most oligonucleotides are further broken down in the small intestine by brush border enzymes such as 5'-nucleotidase and alkaline phosphatase to nucleotides and nucleosides (3). Nucleosides may be further broken down by nucleosidases to yield organic purine and pyrimidine bases which can also be absorbed; no digestion of DNA takes place in the colon (22,23). Nucleosides (organic base plus pentose sugar) which are the major form absorbed at the brush border of enterocytes and are taken up by three Na^+ linked concentrative transporter (CNT1, CNT2, and CNT3) proteins coded for by SLC28 genes; equilibrative (SLC29) transporters located on the basolateral membrane allow nucleosides to exit and reach the blood (8,44). This transport system also absorbs a number of nucleoside analogs used as antiviral drugs.

3. Other aspects of DNase 1

DNase 1 is used in the laboratory technique of DNA footprinting to determine the sequence, affinity and kinetics of DNA binding proteins (9). Footprinting is a protection assay in which the action of the cleavage agent is blocked by the binding of a protein ligand at a specific site. DNase 1 is also used to degrade DNA in a variety of techniques such as preparing RNA for RT-PCR analysis.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of anti-nuclear antibodies (ANA) directed against naked DNA and nucleosomes. It includes widespread inflammation of skin, joints and kidneys with many patients showing glomerulonephritis. DNase 1 participates in the clearance of DNA-protein complexes and may prevent the formation of autoantibodies. Patients with SLE and glomerulonephritis show reduced levels of serum DNase 1 and this reduction may play a role in the pathogenesis of inflammation. In another study, two out of twenty patients with SLE showed a mutation in DNase 1 blocking its synthesis (43). Because aberrant rates of apoptosis and an increase in circulating chromatin have been reported in SLE, DNase 1 may be necessary to prevent production of ANA leading to SLE. DNase 1 deficient mice with aging show increased ANA, glomerulonephritis and death (26). In support of this concept, a number of human genetic variants including missense, nonsense, and in frame variants that reduce DNase1 activity have been identified as genetic risk factors for autoimmune diseases including SLE (39).

Clinically, recombinant human DNase 1 is used to reduce the viscosity of sputum in patients with cystic fibrosis transforming it from a gel to a liquid (31,40). Lack of or reduced DNase activity has also been associated with myocardial infarction (MI) suggesting a role for NETs (neutrophil extracellular traps) containing DNA and histones in cardiac thrombosis (20). Treatment of mice, with experimental acute MI, with recombinant DNase 1 reduced the infarct size (30).

4. Tools for the study of DNase1

a. Assay of DNase1 - There are a number of DNase assays based on its hydrolytic activity against DNA. In zymograms, DNA and ethidium bromide are present in a gel and the cleared area around a well appears black (25). Other assays monitor the release of flurophore labelled DNA (10). These activity assays

require the use of an inhibitor for DNase1 to separate out DNase1 activity.

b. Antibodies - Antibodies have been raised against human and animal DNase1 protein and are available from Thermo Fisher Scientific and Abcam that are stated to be suitable for Western blotting and immunolocalization.

c. Genetic models - Mice with gene deletion of DNase 1 have been prepared and described (26,27).

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