PANCREAPEDIA
Exocrine Pancreas Knowledge Base

APA AMERICAN PANCREATIC ASSOCIATION

MOLECULE PAGE

GP-2

John A Williams

From the Department of Molecular and Integrative Physiology, The University of Michigan, Ann Arbor, Michigan 48109-0622

e-mail: jawillms@umich.edu

Version 1.0, December 9, 2015 [DOI: pending]

Gene ID: GP2

1. General

GP-2 also known as ZAP75 is a phosphatidylinositol linked glycoprotein (31) present on the inner surface of the pancreatic zymogen granule (ZG). It was first recognized by MacDonald and Ronzio (26) as the major protein species in ZG membranes (ZGM) and was also noted to stain with PAS reagent. It had a molecular mass about 80 kDa although in various species it varies from 75 to 90 kDa. Its name comes from a subsequent study where pancreatic tissue slices were labeled with ^{3}H-glucosamine and ZGM isolated and electrophoresed. Three major glycoprotein peaks were noted and named in order of increasing mobility GP-1 (120 kDa), GP-2 (74 kDa) and GP3 (52 kDa) (36). Other topological studies showed that GP-2 was present on the inner surface of the membrane (4, 24). GP-2 is the most abundant ZGM protein accounting for 25-40 percent of total protein in different species. Immunolocalizatiom showed GP-2 present on the apical plasma membrane as well as the ZG (2) **(Figure 1)**. Lebel and Beattie and Fukuoka et al showed that GP-2 is linked to the membrane through a glycophophatidyl inositol (GPI) bond. This was based in part on its release from ZGM treated with a PI-specific PLC (12, 22). GP-2 was also shown to be homologous to Tamm-Horsfall protein, a GPI linked protein produced in kidney and excreted in urine (37).

GP-2 is present in pancreatic juice and makes up 5-8% of unstimulated juice protein in the rat and pig (38). Much of the released protein forms fibrils or aggregates and is sedimentable (3). However, it has lost its hydrophobicity as determined by Triton X-114 partitioning and contains a inositol 1,2-(cyclic)monophosphate residue (30, 32). Thus, the GP2 form present in pancreatic juice appears to have lost its GP1 modifiation throught the action of a PLC. However, since there is no PI-specific PLC present in ZGM, it may have to enter the apical acinar cell membrane to undergo processing. This could then explain the presence of significant amounts of GP-2 in basal pancreatic juice. Some GP-2 is also present in ZG content (16) and smaller cleaved forms are also present in pancreatic juice, implying proteolytic cleavage as well. However, where such processing takes place is not clear. Stimulation with secretin has no effect on GP-2 output in pancreatic juice while stimulation with caerulein or carbachol increases secretion, but with a slower time course than the secretion of digestive enzymes (21). GP-2 is also present in ductal proteinaceous plugs found in chronic pancreatitis (10).

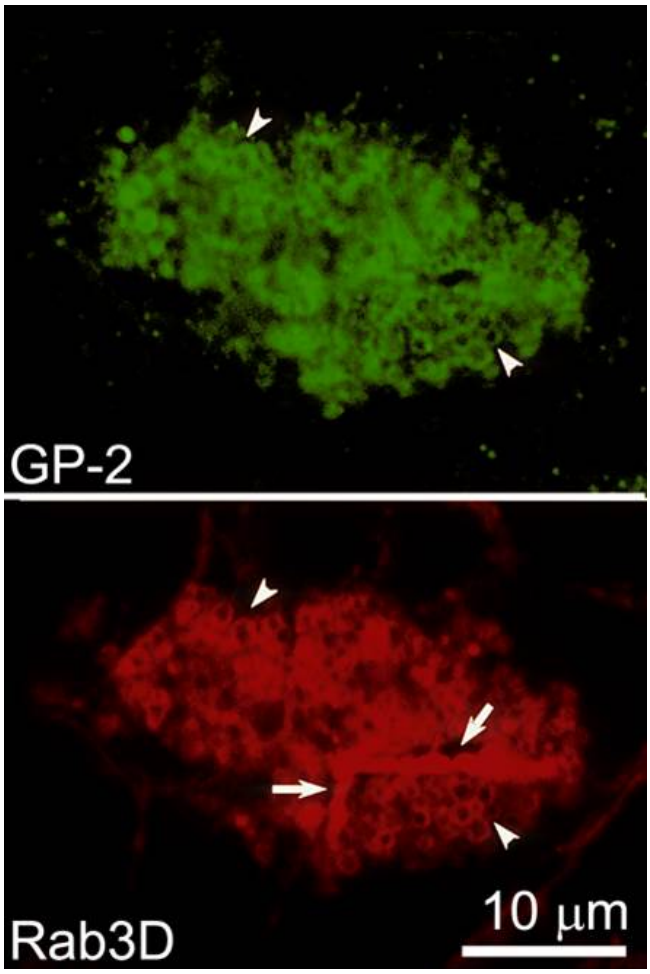

Figure 1. Immunoflourescence localization of GP-2 and Rab3D in rat pancreatic acinar cells. Rab3D localization is restricted to ZG while GP-2 is present both in granules (arrowhead) and acinar lumen (arrows). At this resolution it is not possible to determine if it is on the luminal membrane or in the lumen (27).

The complete nucleotide and amino acid sequence of GP-2 was determined initially by molecular cloning technique from peptide sequence for rat, dog and humans (12, 13, 18, 43). The cDNA encodes a protein of 530 aa in humans with 67% identity to rat and 72% to dog GP-2

The primary sequence encodes 10 (human) or 8 (dog) Asn-linked glycosylation sites. This is consistent with data for in vitro translation by reticulocyte lysate that yields a protein of 55 kDa and treatment with N-glycanase which reduces the molecular mass of canine GP-2 from 75 kDa to 52 kDa (12, 18). Rat GP-2 has been shown to possess 5 or 6 N-linked high mannose carbohydrate chains (16). The primary protein sequence is also post-translationally glycosylated on its carboxyl terminal to a GPI moiety in the membrane during passage through the Golgi to reach the ZG (11, 13, 17). This GPI moiety contains a phosphoethanolamine linker attached to the protein, a 4-5 sugar core and a phosphatidylinositol whose hydrocarbon chains insert into the membrane.

2. Function in the Pancreas

The function of GP-2 remains unknown with attention to a role in the pancreas or downstream in the pancreatic juice or small intestinal lumen. The first suggested role was in the packaging of ZG. In general, secretory granule formation involves an acidic pH-dependent aggregation of secretory proteins within the trans golgi network (TGN) followed by binding of the aggregate to the membrane of the TGN and the pinching off of immature secretory granules (41). In the acinar cell, digestive enzyme proteins can aggregate along with GP-2. It has been hypothesized that GP-2 in conjunction with proteoglycan forms a matrix on the luminal side of the ZGM that can bind the aggregated protein (19, 23, 37). Isolated ZGs contain such a submembranous matrix which contains syncollin and ZGp16 as well as GP-2 (39). In support of a role for this matrix, inhibition of GPI anchor biosynthesis led to impaired granule formation (40). However, in embryonic pancreas and AR42J cells, when granule formation is increased, there is no change in GP-2 (7). Most directly, in GP-2 knockout mice there was no phenotype and granules were formed and digestive enzymes secreted normally (46). However, in the mouse, GP-2 is less abundant than other species and a different protein, muclin, a sulfated glycoprotein,may have become the primary sorting receptor (5, 6).

A second postulated role for GP-2 is in regulating endocytosis of exocytosed ZG membrane. In this

model developed by Freedman, Kern and Scheele, the high bicarbonate and alkaline pH of the duct lumen activates a PI-PLC to cleave GP-2 and other PI linked proteins and their release activates apical membrane endocytosis (8, 9). Evidence for this model is that endocytosis is pH dependent and inhibited at pH 6.0 while addition of PI-PLC activates endocytosis even at pH 6.0. Latter it was observed that GP-2 is present in the acinar cell apical membrane and associated with Src family member Tyr kinases (29). However, since GP-2 expression in AR42J cells did not affect the secretory process or endocytosis (44) a role for GP-2 in vesicular trafficking remains doubtful.

A possible connection of GP-2 to pancreatic disease has also been explored. GP-2 is elevated in the plasma of rats with experimental pancreatic and humans with acute pancreatitis and appears to stay elevated longer than amylase or lipase (14, 25). In one study chronic ethanol feeding decreased GP-2 protein in the pancreas (1). Another study evaluated GP-2 for mutations in chronic pancreatitis but the frequency of mutations was similar to the normal population (42).

3. Role in Intestinal Disease

Recent studies have raised the question of whether GP-2 plays a role after pancreatic juice enters the intestine. One line of evidence began with the showing that GP-2 binds E. Coli that express Type 1 fimbria (45). Recently GP-2 was found to be expressed on M cells, a specialized antigen-transporting cell present in the gut epithelium overlying lymphoid follicles (15, 28). In this location it serves as a bacterial uptake mechanism for gram negative bacteria. However, the relation of this function to pancreatic GP-2 is unclear.

A second area is the role of autoantibodies to GP-2 found in GI disease. About 30% of patients with Crohn's disease and 8% with ulcerative colitis have serum pancreatic autoantibodies and GP-2 is the major pancreatic antigen (33, 34). These antibodies are both IgG and IgA. IgA antibodies against GP-2 have also been reported from patients with coeliac disease (20, 35).The antibodies disappeared following a gluten free diet similar to other autoantibodies in coeliac disease. How these antibodies relate to the disease, however, is not clear.

4. Tools for Study

a. Antibodies. Our studies used a monoclonal Ab to GP-2 developed by Anson Lowe and described in (24). Currently a number of Ab are available commercially including a Atlas antipeptide antibody available from Sigma specific for human GP-2, multiple goat antibodies available from Santa Cruz, and other AB from Novus, GeneTex and OriGene. All are raised against human sequence and would need testing in rats or mice.

b. Elisa Kits. Kits stated to react with dog, human, mouse and rat GP-2 are available from Antibodies on line but have not been tested by us.

5. References

1. **Apte MV,Norton ID,Haber PS,Korsten MA,McCaughan GW,Pirola RC, et al.** Chronic ethanol administration decreases rat pancreatic GP-2 content. *Biochim Biophys Acta* 1336(1): 89-98,1997. PMID: 9271254.
2. **Beaudoin AR,Grondin G and Laperche Y**. Immunocytochemical localization of gamma-glutamyltranspeptidase, GP-2 and amylase in the rat exocrine pancreas: the concept of zymogen granule membrane recycling after exocytosis. *J Histochem Cytochem* 41(2): 225-233,1993. PMID: 7678269.
3. **Beaudoin AR,St-Jean P and Grondin G**. Ultrastructural localization of GP-2 in acinar cells of pancreas: presence of GP-2 in endocytic and exocytic compartments. *J Histochem Cytochem* 39(5): 575-588,1991. PMID: 2016510.

4. **Chen X, Ulintz PJ, Simon ES, Williams JA, Andrews PC.** Global topology analysis of pancreatic zymogen granule membrane proteins. *Mol Cell Proteomics* 7(12):2323-2336, 2008. PMID: 18682380.
5. **De Lisle RC,Norkina O,Roach E and Ziemer D**. Expression of pro-Muclin in pancreatic AR42J cells induces functional regulated secretory granules. *Am J Physiol Cell Physiol* 289(5): C1169-1178,2005. PMID: 15987769.
6. **De Lisle RC and Ziemer D**. Processing of pro-Muclin and divergent trafficking of its products to zymogen granules and the apical plasma membrane of pancreatic acinar cells. *Eur J Cell Biol* 79(12): 892-904,2000. PMID: 11152281.
7. **Dittie A and Kern HF**. The major zymogen granule membrane protein GP-2 in the rat pancreas is not involved in granule formation. *Eur J Cell Biol* 58(2): 243-258,1992. PMID: 1385123.
8. **Freedman SD,Kern HF and Scheele GA**. Apical membrane trafficking during regulated pancreatic exocrine secretion--role of alkaline pH in the acinar lumen and enzymatic cleavage of GP-2, a GPI-linked protein. *Eur J Cell Biol* 65(2): 354-365,1994. PMID: 7536674.
9. **Freedman SD,Kern HF and Scheele GA**. Cleavage of GPI-anchored proteins from the plasma membrane activates apical endocytosis in pancreatic acinar cells. *Eur J Cell Biol* 75(2): 163-173,1998. PMID: 9548373.
10. **Freedman SD,Sakamoto K and Venu RP**. GP-2, the homologue to the renal cast protein uromodulin, is a major component of intraductal plugs in chronic pancreatitis. *J Clin Invest* 92(1): 83-90,1993. PMID: 8326020.
11. **Fritz BA,Poppel CS,Fei MW and Lowe AW**. Processing of the major pancreatic zymogen granule membrane protein, GP-2. *Pancreas* 24(4): 336-343,2002. PMID: 11961485.
12. **Fukuoka S,Freedman SD and Scheele GA**. A single gene encodes membrane-bound and free forms of GP-2, the major glycoprotein in pancreatic secretory (zymogen) granule membranes. *Proc Natl Acad Sci U S A* 88(7): 2898-2902,1991. PMID: 2011597.
13. **Fukuoka S and Scheele G**. Nucleotide sequence encoding the major glycoprotein (GP-2) of rat pancreatic secretory (zymogen) granule membranes. *Nucleic Acids Res* 18(19): 5900,1990. PMID: 2216794.
14. **Hao Y,Wang J,Feng N and Lowe AW**. Determination of plasma glycoprotein 2 levels in patients with pancreatic disease. *Arch Pathol Lab Med* 128(6): 668-674,2004. PMID: 15163232.
15. **Hase K,Kawano K,Nochi T,Pontes GS,Fukuda S,Ebisawa M, et al.** Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature* 462(7270): 226-230,2009. PMID: 19907495.
16. **Havinga JR,Slot JW and Strous GJ**. Membrane detachment and release of the major membrane glycoprotein of secretory granules in rat pancreatic exocrine cells. *Eur J Cell Biol* 39(1): 70-76,1985. PMID: 4085502.
17. **Havinga JR,Strous GJ and Poort C**. Biosynthesis of the major glycoprotein associated with zymogen-granule membranes in the pancreas. *Eur J Biochem* 133(2): 449-454,1983. PMID: 6852052.
18. **Hoops TC and Rindler MJ**. Isolation of the cDNA encoding glycoprotein-2 (GP-2), the major zymogen granule membrane protein. Homology to uromodulin/Tamm-Horsfall protein. *J Biol Chem* 266(7): 4257-4263,1991. PMID: 1999417.
19. **Jacob M,Laine J and LeBel D**. Specific interactions of pancreatic amylase at acidic pH. Amylase and the major protein of the zymogen granule membrane (GP-2) bind to immobilized or polymerized amylase. *Biochem Cell Biol* 70(10-11): 1105-1114,1992. PMID: 1284286.
20. **Laass MW,Rober N,Range U,Noss L,Roggenbuck D and Conrad K**. Loss and Gain of Tolerance to Pancreatic Glycoprotein 2 in Celiac Disease. *PLoS One* 10(6): e0128104,2015. PMID: 26047356.
21. **Laforest L,St-Jean P and Beaudoin AR**. A unique secretory behavior for GP-2 in the exocrine pancreas. *Biochem Biophys Res Commun* 184(2): 888-892,1992. PMID: 1575758.
22. **LeBel D and Beattie M**. The major protein of pancreatic zymogen granule membranes (GP-2) is anchored via covalent bonds to phosphatidylinositol. *Biochem Biophys Res Commun* 154(2): 818-823,1988. PMID: 2456764.
23. **Leblond FA,Viau G,Laine J and Lebel D**. Reconstitution in vitro of the pH-dependent aggregation of pancreatic zymogens en route to the secretory granule: implication of GP-2. *Biochem J* 291 (Pt 1): 289-296,1993. PMID: 8471046.
24. **Lewis DS,MacDonald RJ,Kronquist KE and Ronzio RA**. Purification and partial characterization of an integral membrane glycoprotein from zymogen granules of dog pancreas. *FEBS Lett* 76(1): 115-120,1977. PMID: 852598.
25. **Lowe AW,Luthen RE,Wong SM and Grendell JH**. The level of the zymogen granule protein GP-2 is elevated in a rat model for acute pancreatitis. *Gastroenterology* 107(6): 1819-1827,1994. PMID: 7525398.
26. **MacDonald RJ and Ronzio RA**. Comparative analysis of zymogen granule membrane polypeptides. *Biochem Biophys Res Commun* 49(2): 377-382,1972. PMID: 4640364.

27. **Ohnishi H1, Ernst SA, Wys N, McNiven M, Williams JA.** Rab3D localizes to zymogen granules in rat pancreatic acini and other exocrine glands. *Am J Physiol* 271:G531-G538, 1996. PMID: 8843780.
28. **Ohno H and Hase K**. Glycoprotein 2 (GP-2): grabbing the FimH bacteria into M cells for mucosal immunity. *Gut Microbes* 1(6): 407-410,2010. PMID: 21468225.
29. **Parker EM,Zaman MM and Freedman SD**. GP-2, a GPI-anchored protein in the apical plasma membrane of the pancreatic acinar cell, co-immunoprecipitates with src kinases and caveolin. *Pancreas* 21(3): 219-225,2000. PMID: 11039464.
30. **Paul E,Leblond FA and LeBel D**. In resting conditions, the pancreatic granule membrane protein GP-2 is secreted by cleavage of its glycosylphosphatidylinositol anchor. *Biochem J* 277 (Pt 3): 879-881,1991. PMID: 1651706.
31. **Paulick MG and Bertozzi CR**. The glycosylphosphatidylinositol anchor: a complex membrane-anchoring structure for proteins. *Biochemistry* 47(27): 6991-7000,2008. PMID: 18557633.
32. **Rindler MJ and Hoops TC**. The pancreatic membrane protein GP-2 localizes specifically to secretory granules and is shed into the pancreatic juice as a protein aggregate. *Eur J Cell Biol* 53(1): 154-163,1990. PMID: 2076702.
33. **Roggenbuck D,Hausdorf G,Martinez-Gamboa L,Reinhold D,Buttner T,Jungblut PR, et al.** Identification of GP-2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut* 58(12): 1620-1628,2009. PMID: 19549613.
34. **Roggenbuck D,Reinhold D,Werner L,Schierack P,Bogdanos DP and Conrad K**. Glycoprotein 2 antibodies in Crohn's disease. *Adv Clin Chem* 60: 187-208,2013. PMID: 23724745.
35. **Roggenbuck D,Vermeire S,Hoffman I,Reinhold D,Schierack P,Goihl A, et al.** Evidence of Crohn's disease-related anti-glycoprotein 2 antibodies in patients with celiac disease. *Clin Chem Lab Med* 53(9): 1349-1357,2015. PMID: 25411995.
36. **Ronzio RA,Kronquist KE,Lewis DS,MacDonald RJ,Mohrlok SH and O'Donnell JJ, Jr.** Glycoprotein synthesis in the adult rat pancreas. IV. Subcellular distribution of membrane glycoproteins. *Biochim Biophys Acta* 508(1): 65-84,1978. PMID: 629968.
37. **Scheele GA,Fukuoka S and Freedman SD**. Role of the GP-2/THP family of GPI-anchored proteins in membrane trafficking during regulated exocrine secretion. *Pancreas* 9(2): 139-149,1994. PMID: 8190715.
38. **Scheffer RC,Poort C and Slot JW**. Fate of the major zymogen granule membrane-associated glycoproteins from rat pancreas. A biochemical and immunocytochemical study. *Eur J Cell Biol* 23(1): 122-128,1980. PMID: 7460957.
39. **Schmidt K,Dartsch H,Linder D,Kern HF and Kleene R**. A submembranous matrix of proteoglycans on zymogen granule membranes is involved in granule formation in rat pancreatic acinar cells. *J Cell Sci* 113 (Pt 12): 2233-2242,2000. PMID: 10825295.
40. **Schmidt K,Schrader M,Kern HF and Kleene R**. Regulated apical secretion of zymogens in rat pancreas. Involvement of the glycosylphosphatidylinositol-anchored glycoprotein GP-2, the lectin ZG16p, and cholesterol-glycosphingolipid-enriched microdomains. *J Biol Chem* 276(17): 14315-14323,2001. PMID: 11152672.
41. **Tooze SA**. Biogenesis of secretory granules in the trans-Golgi network of neuroendocrine and endocrine cells. *Biochim Biophys Acta* 1404(1-2): 231-244,1998. PMID: 9714820.
42. **Witt H,Rosendahl J,te Morsche RH,Santhosh S,Chacko A,Schulz HU, et al.** Mutational analysis of the gene encoding the zymogen granule membrane glycoprotein 2 (GP-2) in patients with chronic pancreatitis. *Pancreas* 39(2): 188-192,2010. PMID: 19959969.
43. **Wong SM and Lowe AW**. Sequence of the cDNA encoding human GP-2, the major membrane protein in the secretory granule of the exocrine pancreas. *Gene* 171(2): 311-312,1996. PMID: 8666297.
44. **Yu S,Hao Y and Lowe AW**. Effects of GP-2 expression on secretion and endocytosis in pancreatic AR4-2J cells. *Biochem Biophys Res Commun* 322(1): 320-325,2004. PMID: 15313209.
45. **Yu S and Lowe AW**. The pancreatic zymogen granule membrane protein, GP-2, binds Escherichia coli Type 1 fimbriae. *BMC Gastroenterol* 9: 58,2009. PMID: 19627615.
46. **Yu S,Michie SA and Lowe AW**. Absence of the major zymogen granule membrane protein, GP-2, does not affect pancreatic morphology or secretion. *J Biol Chem* 279(48): 50274-50279,2004. PMID: 15385539.