GP-3, a Newly Characterized Glycoprotein on the Inner Surface of the Zymogen Granule Membrane, Undergoes Regulated Secretion*

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We have recently reported the cloning of the rat zymogen granule membrane glycoprotein GP-3 and the related pancreatic secretory lipase (Wishart, M. J., Andrews, P. C., Nichols, R., Blevins, G. T., Logsdon, C. D., and Williams, J. A. (1993) J. Biol. Chem. 268, 10303-10311). Specific antipeptide antibodies were generated against both GP-3 and secretory lipase and used for the biochemical and physiological characterization of GP-3. Western blotting confirmed that GP-3 was found exclusively in zymogen granule membranes and was absent from zymogen granule content which contains the majority of secretory lipase. Extraction of zymogen granule membranes with Triton X-114 showed GP-3 to be significantly more hydrophobic than lipase. The GP-3 amino acid sequence contains one potential N-linked glycosylation site at Asn-336. The loss of concanavalin A labeling after both chemical deglycosylation with trifluoromethanesulfonic acid and enzymatic deglycosylation with N-glycanase showed GP-3 to possess a small N-linked oligosaccharide side chain. Digestion of intact and permeabilized zymogen granules with nonspecific protease Pronase localized GP-3 to the inner surface of zymogen granule membranes. Since GP-3 is resident on the inner surface of the zymogen granule membrane, it should appear on the outer cellular surface after exocytosis. Although membrane attachment of GP-3 was resistant to treatment with phosphatidylinositol-specific phospholipase C, we observed that GP-3 is released into the pancreatic juice and that secretion of GP-3 was greatly enhanced by cholecystokinin.

The main function of pancreatic acinar cells is the synthesis and regulated secretion of digestive enzymes. These enzymes or proenzymes are stored in the zymogen granules which are located in the apical pole of the cell. Stimulation with secretagogues such as cholecystokinin and carbachol triggers a series of events resulting in fusion of the zymogen granule membrane with the apical plasma membrane and subsequent release of the zymogen granule content into the pancreatic duct. Following exocytosis, the granule membrane is retrieved by endocytosis and recycled (Scheele and Kern, 1993). Although early events in stimulus-secretion coupling and second messenger generation in acini are well understood (Williams and Yule, 1993), little is known about the processes involved in fusion of zymogen granule membranes with the apical plasma membrane and endocytosis.

It seems likely that the protein components of the zymogen granule membrane will play an important role both in granule formation and secretion. Earlier studies have shown that the protein composition of zymogen granule membranes from different species including rat (Ronzio et al., 1978; Paquet et al., 1982; Beaudoin et al., 1983) and pig (Lebel and Beattie, 1984a) is relatively simple. Using SDS-PAGE1 for separation of zymogen granule membrane proteins together with a variety of labeling methods, 8-10 different proteins have been described in purified zymogen granule membranes ranging in molecular mass from 14,000 to 140,000 Da. Staining with periodic acid Schiff-base reagent (PAS) identified three zymogen granule membrane proteins of 120, 50, and 52 kDa as glycoproteins which were named GP-(glycoprotein) 1, 2, and 3 (Ronzio et al., 1978) respectively. GP-2 was found to be most abundant, accounting for up to 30-40% of the zymogen granule membrane protein in some studies.

GP-2 was the first zymogen granule protein to be cloned and characterized at the molecular level as a glycosophosphatidylinositol-linked glycoprotein (Fukuoka and Scheele, 1990; Fukuoka et al., 1991; Hoops and Rindler, 1991). We have recently cloned GP-3 and have shown that it is a membrane bound glycoprotein homologous to but distinct from pancreatic secretory lipase (Wishart et al., 1993). Others have cloned the mouse and human homologs of GP-3 and shown that they possess lipase activity which is relatively colipase-independent (Grusby et al., 1990; Giller et al., 1992). To further compare and characterize GP-3 and lipase, we have generated specific affinity-purified antipeptide antibodies against both proteins. In this study we show that GP-3 is localized solely to the inner surface of zymogen granule membranes, partitions as a membrane protein upon detergent extraction, and contains a small N-linked oligosaccharide chain. Although GP-3 is not found in granule content preparations, it is secreted with the pancreatic juice, similar to lipase, and this secretion is strongly regulated by cholecystokinin.

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1 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; MOPS, 3-(N-morpholino)propanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; PI-PLC, phosphatidylinositol-specific phospholipase; GP, glycoprotein; TFMS, trifluoromethanesulfonic acid; PMSE, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; ConA, concanavalin A; ZG, zymogen granule; ZGM, zymogen granule membranes; ZGC, zymogen granule content; CCK, cholecystokinin; WGA, wheat germ agglutinin; GPI, glycan-phosphatidylinositol.
MATERIAL AND METHODS

Chemicals—Trifluoromethanesulfonic acid (TFMS), anisole, ethanolamine, tritylamine, 4-A molecular sieves, and pyridine were purchased from Aldrich; detection reagents for enhanced chemiluminescence (ECL) and horseradish peroxidase-coupled anti-mouse and anti-rabbit antibodies were from Amersham Corp.; SDS, polysacrylamide, Affi-Gel 10 active ester-apoagarose, and proteinase were from Bio-Rad; bovine serum albumin from fraction V was from the Collaborative Research. Recombinant murine IFN-γ was from Genzyme (Cambridge, MA); Triton X-114 was from Pierce Chemical Co.; Pronase was from Boehringer Mannheim. Horseradish peroxidase-coupled lectins and all other chemicals were obtained from Sigma.

Purification of Zymogen Granules and Zymogen Granule Membranes—Purified zymogen granules and zymogen granule membranes were prepared by modification of previously published procedures (De Lisle et al., 1984; Burnham et al., 1985). Briefly, pancreata from male Sprague-Dawley rats were homogenized in buffer containing 250 mM sucrose, 5 mM Tris, pH 7.0, 0.1 mM magnesium sulfate, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and centrifuged at 200 x g for 5 min to remove cellular debris. The supernatants were then centrifuged at 2000 x g for 15 min, and the resulting pellet containing mainly mitochondria and zymogen granules was mixed with 50% sucrose and centrifuged at 100,000 x g for 90 min, the white band near the bottom of the tube containing highly purified zymogen granules was collected and washed in 300 mM sucrose, 20 mM MES, pH 5.5, 1 mM EDTA, 0.1 mM MgSO₄, and 0.1 mM PMSF. For preparation of purified zymogen granule membranes, granules were osmotically lysed in 150 mM sodium acetate, 10 mM MOPS, pH 7.0, 0.1 mM magnesium sulfate, and 0.1 mM PMSF. After centrifugation at 25,000 x g for 90 min, the white band near the bottom of the tube containing highly purified zymogen granules was collected and washed in 300 mM sucrose, 20 mM MES, pH 5.5, 1 mM EDTA, 0.1 mM MgSO₄, and 0.1 mM PMSF. For preparation of purified zymogen granule membranes, granules were osmotically lysed in 150 mM sodium acetate, 10 mM MOPS, pH 7.0, 0.1 mM magnesium sulfate, and 0.1 mM PMSF. Affi-Gel (10 ml) was incubated overnight at room temperature with 5 pl of ethanolamine for 1 h. The beads were washed three times with 50 ml of phosphate-buffered saline (1X PBS) and eluted with 10 ml of elution buffer, pH 8.5, 0.5% Triton X-114, and then by addition of 0.5 mM of elution buffer. After washing with 50 ml of 5X PBS, the beads were eluted with 10 ml of 10X PBS containing, pH 2.5, 0.5% Triton X-114. Ten micrograms of protein was loaded on a 10% gel for electrophoresis and transferred to nitrocellulose from the gel (Erickson et al., 1982; Wang et al., 1989). Two-dimensional polyacrylamide gel electrophoresis was performed as described (Wagner et al., 1990). Peptides above were synthesized at the University of Michigan Protein Structure Core Facility. Each multiple antigenic peptide was dissolved in water-free dimethyl sulfoxide and 100 µl of peptide was added to 20 ml of water-free dimethyl sulfoxide and 100 µl of water-free triethylamine. The reaction was quenched with 500 µl of ethanolamine for 1 h. The beads were washed three times with 50 ml of dimethyl sulfoxide and several times with 1 mM acetic acid and distilled water and stored in 0.05% sodium azide at 4 °C. For affinity purification of the antigen, 1 ml of peptide-coupled beads was poured into polyprop chromatography columns (Bio-Rad) following by equilibration with 5X phosphate-buffered saline (1X PBS = 137 mM NaCl, 2.7 mM KCl, 10.1 mM Na₂HPO₄, and 1.5 mM KH₂PO₄). One milliliter of antigen was mixed with 1 ml of 10X PBS and passed over the column. After washing with 50 ml of 5X PBS, antibodies were eluted with 10 ml of 10X PBS containing, pH 8.5, 0.5% Triton X-114 and 0.1% SDS. The eluate was concentrated to 100 µl by vacuum dialysis. For immunoblotting of the antigen, 10 µl of antibody solution was loaded on a 10% gel and transferred to nitrocellulose. Detection of glycoproteins was then performed as above.

Probing with Antibodies and Lectins—Ninety microgram of TFMS was carried out as described previously (Horvath et al., 1989). To remove salts, 60 µg of zymogen granule membranes (ZGM) in 20 mM Tris-HCl, 20 mM NaCl, 1% bovine serum albumin added instead of ovalbumin. Strips were washed and then probed with the indicated concentrations of antibodies for 1 h. Labeled proteins were visualized by enhanced chemiluminescence following the manufacturers procedures, by exposing the membranes to X-ray film. For lectin staining, membranes were blocked overnight in TBS with 5% bovine serum albumin and then probed with the indicated lectins coupled to horseradish peroxidase at a concentration of 1 µg/ml for 4 h. Detection of glycoproteins was then performed as above.

Deglycosylation Experiments—Chemical deglycosylation using TFMS was carried out as described previously (Horvath et al., 1989). To remove salts, 60 µg of zymogen granule membranes (ZGM) in 20 mM Tris-HCl, 20 mM NaCl, 1% bovine serum albumin added instead of ovalbumin. Strips were washed and then probed with the indicated concentrations of antibodies for 1 h. Labeled proteins were visualized by enhanced chemiluminescence following the manufacturers procedures, by exposing the membranes to X-ray film. For lectin staining, membranes were blocked overnight in TBS with 5% bovine serum albumin and then probed with the indicated lectins coupled to horseradish peroxidase at a concentration of 1 µg/ml for 4 h. Detection of glycoproteins was then performed as above.
lanes 2 and 4 were added to the serum 30 min prior to probing. The effect of dimethyl sulfoxide alone, required to dissolve the peptides, is shown in lanes 5–8. Antibodies. Detection was performed with 1:5000 horseradish peroxidase-coupled anti-rabbit IgG. Three sera were incubated with 10 pg/ml of the GP-3 peptide A and the lipase peptide C, to react best with native denatured GP-3 and lipase, which appears as a contamination in our zymogen granule membrane preparations, the affinity-purified antibodies were incubated with 10 μg/ml of either the GP-3 peptide A and the lipase peptide C and subsequently tested for remaining reactivity. The reactivity of the anti-GP-3 antibody was only blocked by the peptide A and not by the peptide C (Fig. 1, lanes 3 and 4), whereas the anti-lipase antibody was blocked by peptide C but not the peptide A (Fig. 1, lanes 7 and 8). The presence of 1% dimethyl sulfoxide, required for dissolving the peptides, somewhat diminished the ECL signal but still allowed visualization of both secretory lipase and GP-3 by their respective antibodies (Fig. 1, lanes 2 and 6). Only the affinity-purified antibodies were used for all further studies. The specificity of the antibodies was also confirmed by Western blots of two-dimensional gel electrophoresis of zymogen content and purified zymogen granule membranes which revealed GP-3 to be present only in the membranes (data not shown). The antisera clearly distinguish between the two proteins and confirmed our previous results (Wishart et al., 1993), that GP-3 is only found in zymogen granule membranes.

**GP-3 is a Glycoprotein with a Small N-Linked Oligosaccharide Chain**—Concanavalin A labeled mainly one band at 53 kDa in purified zymogen granule membranes (Fig. 2, lane 2; Fig. 3, lane 6). When these membranes were treated with TFMS and subjected to Western transfer and lectin staining, the 53-kDa band was no longer labeled by ConA (Fig. 2, lane 1). Stripping these same nitrocellulose membranes, thereby completely removing the lectins (not shown), and reprobing with antibodies against both GP-3 and GP-2 showed that both proteins could still be detected after TFMS treatment (Fig. 2, lane 3). Using the same nitrocellulose membrane for both the lectin and the antibody probing allowed the identification of the 53-kDa band visualized by ConA staining as GP-3. TFMS treatment reduced the molecular mass of GP-2 from 80 to 60 kDa, whereas the molecular mass of GP-3 was only slightly reduced (Fig. 2, lanes 3 and 4). To further characterize the glycosylation of GP-3, we have treated zymogen granule membranes with N-glycanase. Using WGA, one band of 80 kDa and two weak bands of lower apparent molecular mass were observed in untreated membranes (Fig. 3, lane 2). All the bands visualized with either lectin disappeared after N-glycanase treatment (Fig. 3, lanes 1 and 5). Reprobing with anti-GP-3 (Fig. 3, lanes 7 and 8) or GP-2 (Fig. 3, lanes 3 and 4) antibodies showed that...
both proteins could still be detected after N-glycanase treatment. The shifts in molecular mass due to N-glycanase were identical to the results observed with TFMS. We could also clearly identify the 53- and 80-kDa bands recognized by ConA and WGA as GP-3 and GP-2, respectively.

GP-3 Partitions Evenly into the Detergent and Aqueous Phase after Triton X-114 Extraction—To further characterize the hydrophobic properties of GP-3, purified zymogen granule membranes were extracted with Triton X-114 and subjected to Western analysis. Consistent with previous work, GP-2 almost exclusively partitioned into the detergent phase (Fig. 4, lanes 1–3) (Havinga et al., 1985; LeBel and Beattie, 1988). In contrast, GP-3 distributed equally into the aqueous and detergent phases (Fig. 4, lanes 4–6), whereas secretory lipase partitioned totally into the aqueous phase (Fig. 4, lanes 7–9). When the two fractions obtained after Triton X-114 extraction were subjected to a second round of detergent extraction, GP-3 from both the aqueous and the detergent phases again separated equally into the aqueous and detergent phases (not shown), indicating that the partitioning between the two phases represents an intrinsic hydrophobic behavior rather than two different forms of GP-3.

GP-3 Is Located on the Inside of Zymogen Granules—Pronase was used to determine the orientation of GP-3. Since Pronase cannot cross lipid bilayer membranes, digestion of intact zymogen granules with Pronase can only affect proteins which are exposed on the outer surface of the granules, whereas content proteins as well as zymogen granule membrane proteins oriented toward the inside remain intact. Permeabilization of the granules with the nonionic detergent Nonidet P-40 allows the Pronase to enter the granules for digestion of the heretofore protected proteins. Antibody detection of GP-2, which is known to face the inside of ZG (Ronzo et al., 1978), was lost only when granules were permeabilized prior to digestion. (Fig. 5, lanes 3 and 4). Similarly, using the anti-GP-3 antibody, the 53-kDa signal disappeared only after Pronase digestion of permeabilized granules, localizing GP-3 to the inner granular surface (Fig. 5, lanes 7 and 8). Permeabilization with Nonidet P-40 alone had no effect on the signals.

GP-3 Is Secreted in the Pancreatic Juice and Its Secretion Is Strongly Regulated by CCK—Since GP-3 is a zymogen granule membrane protein oriented toward the inside of the granules, it should appear in the apical plasma membrane facing the outside after membrane fusion and exocytosis have occurred. We therefore tested whether GP-3 is secreted in the pancreatic juice, and if so, whether the secretion is regulated by CCK. Basal protein secretion without CCK stimulation (pre-CCK) was 0.44 mg/30 min in a volume of 8 μl. CCK stimulation increased protein secretion to 4.2 mg/30 min in a volume of 80 μl. Twenty min after CCK infusion had been stopped, secretion (post-CCK) had returned to 0.5 mg of protein/30 min in a volume of 15 μl. Western transfers of juice proteins were probed with anti-lipase as well as anti-GP-3 antibody (lanes 1–4), showing that GP-3 is secreted in the pancreatic juice with an apparent molecular mass of 53 kDa and that its secretion is strongly regulated by CCK similar to the regulation of secretory lipase (compare Fig. 6, Lipase: lanes 1–3 with GP-3: lanes 1–3). A small amount of GP-3 was released into the juice without CCK stimulation, as revealed by longer exposures of the nitrocellulose membranes (not shown). For comparison, samples of zymogen granule content (ZGC), total ZG, and ZGM were also run in parallel showing that GP-3 in the juice is not reduced in apparent molecular mass compared to membrane bound GP-3 and further confirming that GP-3 occurs only in the zymogen granule membrane and, in contrast to secretory lipase, is not present in the content. (Fig. 6, lanes 4–6).

Membrane Attachment of GP-3 Is Resistant to PI-PLC—Similar to GP-2, GP-3 is a ZGM protein secreted into the pancreatic juice. We therefore investigated whether GP-3 might also be anchored to the ZGM via a glycan-phosphatidylinositol moiety as is GP-2. Although incubation of purified ZGM for 1 h
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Fig. 6. GP-3 is secreted in pancreatic juice. Pancreatic juice was collected for 30 min by cannulation of the pancreatic duct of pentobarbital-anesthetized rats prior to (pre-CCK), during (CCK), and after (post-CCK) infusion of 300 pmol/kg h CCK. For Western analysis, the amount of juice secreted within 30 s under the respective conditions was loaded on minigels (A and B, lanes 1–3). For comparison, 4.5 μg each of ZG content (ZGC), total ZG, and ZG membrane (ZGM) proteins were also loaded (lanes 4–6). Membranes were probed with anti-lipase (A) and anti-GP-3 (B) antibodies.

Fig. 7. Membrane attachment of GP-3 is resistant to PI-PLC. Zymogen granule membranes (20 μg) were treated with (lanes 3, 4, 7, and 8) or without (lanes 1, 2, 5, and 6) 0.3 unit/ml PI-PLC for 1 h at 37 °C. ZGM were then centrifuged at 100,000 × g for 30 min and pellets (P) (lanes 1, 3, 5, and 7) and supernatants (SN) (lanes 2, 4, 6, and 8) were separated and subjected to Western analysis with antibodies to both GP-2 (lanes 1–4) and GP-3 (lanes 5–8) as in Fig. 1.

at 37 °C had no apparent effect on both GP-2 and GP-3 (Fig. 7, lanes 1, 2, 5, and 6), GP-2 was effectively cleaved off the ZGM by PI-PLC treatment and appeared in the centrifugation supernatant (lanes 3 and 4). In contrast, GP-3 did not appear in the supernatant after PI-PLC treatment, showing that membrane attachment of GP-3 is resistant to PI-PLC cleavage (lanes 7 and 8).

**DISCUSSION**

Despite an overall 70% amino acid identity (Wishart et al., 1993), detection of GP-3 and lipase by their respective affinity-purified antibodies could be shown. Western analysis thus confirmed earlier findings based on protein staining (Wishart et al., 1993) that GP-3 is found exclusively in zymogen granule membranes but not in zymogen granule content, whereas secretory lipase is found in both preparations.

GP-3 has been identified as a glycoprotein based on PAS staining as well as ConA and WGA labeling (Wishart et al., 1993). In contrast to GP-2, which undergoes a significant reduction in apparent molecular mass of 20 kDa when deglycosylated, only a very small reduction in molecular mass of GP-3 was apparent after chemical deglycosylation or enzymatic deglycosylation with N-glycanase. The shift in apparent molecular mass of GP-2 after deglycosylation from 80 to 60 kDa corresponds well with earlier studies which showed GP-2 to contain at least six oligosaccharide chains (Havinga et al., 1985), as well as cDNA analysis of GP-2 revealing eight potential N-linked glycosylation sites (Fukuoka et al., 1991; Hoops and Rindler, 1991) and predicting a molecular mass of post-translationally unmodified GP-2 of 59 kDa. In contrast, sequence analysis of GP-3 has revealed only one potential N-linked glycosylation site at Asn-336 and a predicted molecular mass of 51 kDa (Wishart et al., 1993), also corresponding well with the data obtained in our deglycosylation experiments. There was no apparent difference in lectin labeling and molecular mass between chemical and enzymatic deglycosylation methods. Together with the cDNA data, this suggests that GP-3 most likely contains a single small N-linked oligosaccharide side chain, most likely at Asn-336. We have not analyzed the sugar composition of this side chain in detail; however, speculations can be made based upon knowledge of N-linked oligosaccharide structure and lectin specificity. The same pentasaccharide core (Manα1-3Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-Asn) is present in all asparagine-linked oligosaccharides (Kornfeld and Kornfeld, 1985; Spicer and Schulte, 1989) and is likely also to be present in GP-3. The strong GP-3 labeling by ConA, which is specific for mannose residues, indicates a high mannose form of N-linked glycosylation, whereas the failure of WGA to visualize GP-3 (Fig. 3, lane 2) suggests that GP-3 glycosylation contains no terminal GlcNAc residues. Together with the apparent small size of the oligosaccharide chain of GP-3, we would thus predict the pentasaccharide core and possibly some additional sugar residues as the structure of the GP-3 oligosaccharide side chain.

GP-3 was identified as a ZGM protein because it could not be washed off the ZGM by KBr and NaCO3 (Wishart et al., 1993). Extraction with the detergent Triton X-114 was consistent with the characterization of GP-3 as a membrane-associated protein (Fig. 4). Triton X-114 is a commonly used tool to characterize membrane proteins (Bordier, 1981; Prive, 1986) and their solubility. In contrast to secretory lipase which totally partitioned into the aqueous phase, GP-3 was found in both the aqueous and the detergent phase. Partitioning of membrane proteins and membrane glycoproteins into both phases has been reported (Prive and Phillips, 1986). This further establishes the membrane protein nature of GP-3 in contrast to the soluble hydrophilic nature of its closely related homologue, secretory lipase. GP-2 has also been reported to partition into both phases upon Triton X-114 extraction (Havinga et al., 1985). However, the GP-2 in the aqueous phase appears to change its character from a hydrophobic membrane-bound to a hydrophilic soluble content protein reduced in apparent molecular mass by 4–5 kDa. The fraction of GP-3 partitioning into the aqueous phase, however, exhibited no change of apparent molecular mass and also did not behave like a soluble hydrophilic protein when subjected to a second round of Triton X-114 extraction (not shown).

Digestion of intact zymogen granules, as well as permeabilized granules, with Pronase showed GP-3 to be resident on the inner surface of the ZGM, similar to GP-2. This further supports the identification of the protein we have described previously as a 53-kDa zymogen granule membrane protein stable by a combination of the lectins ConA and WGA (Wishart et al., 1993), as the 52-kDa rat ZG granule membrane protein (GP-3) described by Ronzio et al., 1978, as judged by its similarity in apparent molecular mass, its glycoprotein nature, and its localization.
Although GP-3 is attached to the inner ZGM surface, we found that GP-3 is secreted into the pancreatic juice and that its secretion is strongly regulated by CCK. Thus GP-3 is yet another secreted ZGM protein. The appearance of GP-3 in the juice, despite its absence from zymogen granule content, raises the question of how it is attached to the ZGM and how it might be released from the membrane during or after secretion. GP-3 lacks an obvious transmembrane domain; however, the replacement of charged residues by hydrophobic amino acids in its C terminus relative to other lipases has been observed (Wisheart et al., 1993). Although a stretch of the carboxyl domain of GP-3 conforms to the recently proposed sequence requirements for glycan-phosphatidylinositol (GPI) anchoring (Coyne et al., 1993), digestion of ZGM with PI-PLC indicates that GP-3 might not be anchored to the plasma membrane by a GPI linkage. The membrane attachment of GP-2 has recently been investigated (LeBel and Beattie, 1988; Rindler and Hoops, 1990; Fukuoka et al., 1992) by Western analysis with an antibody recognizing a PI-PLC-resistant GPI anchor after PI-PLC cleavage. Recognition of GP-2 by this antibody after PI-PLC treatment of ZGM, but not that by another antibody after PI-PLC treatment of ZGM, but not that by another antibody, suggests the presence of PI-PLC-resistant GPI linkage of GP-2. If GP-3 is GPI-linked in the same manner as GP-2, it should have been visualized by this antibody after PI-PLC treatment of ZGM, but such a finding has not been reported. However, a PI-PLC-resistant GPI anchor has been observed in human erythrocyte acetylcholinesterase (Roberts et al., 1988), and the resistance has later been shown to depend on palmitoylation of the inositol residue in the 2 or 3 position (Ferguson, 1992). Thus, we cannot exclude a similar PI-PLC-resistant GPI linkage of GP-3. Besides GPI linkage, there are several other means by which proteins without hydrophobic transmembrane domains can be closely attached to lipid bilayer membranes, including isoprenylation (Schafer and Rine, 1992; Maltese, 1990) and N-terminal myristoylation (Gordon et al., 1991); however, GP-3 lacks the amino acid sequence motifs required for these modifications. Proteins can also be attached to membranes through the addition of fatty acid side chains such as N-linked myristoylation as well as O- or S-linked attachment of palmitic or stearic acid (Chow et al., 1992; Rucker and McGec, 1993). Further studies need to be carried out to understand the mechanism of GP-3 attachment to the ZGM.

In conclusion, we have developed specific antibodies for characterization of both rat pancreatic secretory lipase and GP-3. GP-3 was identified as a ZGM protein on the inner ZGM surface with a PI-PLC-resistant membrane secretary lipase and was further found to possess a small N-linked oligosaccharide chain. We also showed that GP-3 undergoes regulated secretion, although it is not present in zymogen granule content.

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