Heterotrimeric G-protein G_{q/11} Localized on Pancreatic Zymogen Granules Is Involved in Calcium-regulated Amylase Secretion*

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The heterotrimeric G-protein G_{q/11} was identified on pancreatic acinar zymogen granules and its function in calcium-regulated exocytosis was examined. Western blotting showed \( \alpha_{q/11} \), but not \( \alpha_i \) or \( \alpha_o \), to be localized to the zymogen granule membrane along with G-protein \( \beta \)-subunit; all three \( \alpha \) subunits were present in a plasma membrane fraction and the \( \alpha_{q/11} \) signal was 30-fold more enriched in the plasma membrane as compared with granule membrane. Neither CCK receptors nor \( \alpha \) subunits of the sodium pump, both plasma membrane markers were present on granule membranes. Immunohistochemistry of pancreatic lobules showed that \( \alpha_{q/11} \) localized to the zymogen granule-rich apical region of acinar cells together with a much stronger signal at the basolateral plasma membrane. When the substance-P-related peptide GPAnt-2a, an antagonist of \( G_{q/11} \), was introduced into streptolysin-O permeabilized acini to bypass the plasma membrane, the amylase release induced by 10 \( \mu \)M free calcium was potentiated in a concentration-dependent manner. By contrast, another substance-P-related peptide, GPAnt-1, an antagonist of \( G_o \) and \( G_i \), showed no effect on calcium-induced amylase release from permeabilized acini. GPAnt-2a peptide also exerted an inhibitory effect on the total GTPase activity of the purified zymogen granules and a larger inhibitory effect on the GTPase activity of the \( G_{q/11} \) protein immunopurified from zymogen granules. GPAnt-1, however, did not inhibit GTPase activity of either zymogen granules or immunopurified \( G_{q/11} \). These results suggest that GPAnt-2a peptide augmented calcium-induced amylase release from permeabilized acini by inhibiting GTPase activity of the \( G_{q/11} \) protein on zymogen granules. We conclude that \( G_{q/11} \) protein on zymogen granules plays a tonic inhibitory role in calcium-regulated amylase secretion from pancreatic acini.

Regulated exocytosis involves the highly controlled targeting, docking, and fusion of secretory vesicles to the plasma membrane. Studies using cell permeabilization and non-hydrolyzable GTP analogues such as GTP\(^\gamma\)S\(^1\) have indicated that G-proteins play key roles in regulated exocytosis (1–3). Rab proteins, members of the Ras-related small G-protein family, are well known to participate in various steps of intracellular vesicle trafficking including exocytosis (4–7). Isoforms of Rab3 have been localized to secretory granules of neuronal and non-neuronal cells, although their role in exocytosis is still not clear (7–9). Recently, we and others have reported that Rab3D (10) is localized to secretory granules of various tissues including exocrine pancreas and its localization implies that it may be involved in regulated exocytosis (11–13). In addition to small G-proteins, recent evidence suggest that heterotrimeric G-proteins or their isolated \( \alpha \)-subunits also play important roles in intracellular vesicle trafficking and vesicle formation in addition to their classical functions in receptor-coupled signal transduction. For example, a heterotrimeric G-protein(s) was shown to be required in endosome fusion in a cell-free system (14). In LLC-PK1 epithelial cells, when \( G_3 \) was overexpressed on Golgi membranes, constitutive secretion was retarded (15). By using an ADP-ribosylation method, \( G_3 \) as well as \( G_{a_4} \) on Golgi membranes were also demonstrated to participate in the regulation of vesicle formation in PC12 cells (16). In addition, evidence suggests heterotrimeric G-proteins may have a role in regulated exocytosis since \( G_3 \) on secretory granules was recently shown to play an inhibitory role in calcium-stimulated norepinephrine release from chromaffin cells (17, 18). In insulin secreting B-cells, \( G_3 \) on secretory vesicles has been demonstrated to be involved in mastoparan-induced insulin secretion (19). Although \( G_{q/11} \) has recently been localized to Golgi membrane (20), its function in vesicle trafficking is still unknown.

The exocrine pancreas is a model system widely used for studying regulated exocytosis. Several small GTP-binding proteins have been shown to be present on zymogen granules, and two have recently been identified as Rab3D and Rab5 (12, 13, 21). However, little is known about the presence or function of heterotrimeric G-proteins on zymogen granules. Although the existence of a pertussis toxin-sensitive G-protein on pancreatic zymogen granules was previously suggested (22) its identity is still uncertain. In the current work, we have identified a heterotrimeric G-protein(s), which localizes to zymogen granules and appears to participate in regulated exocytosis. We found that both \( \alpha_{q/11} \) and G-protein \( \beta \)-subunit exist on zymogen granules. We also examined the effect of substance-P-related peptides on calcium-induced amylase release from streptolysin-O permeabilized acini. Substance-P-related peptide GPAnt-2a and GPAnt-1 were reported to antagonize \( G_{q/11} \) and \( G_{a_4} \), respectively (23) and thus these peptides are widely used for analyzing the function of heterotrimeric G-proteins on both plasma membranes and secretory vesicles (17, 18, 24).

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‡The abbreviations used are: GTP\(^\gamma\)S, guanosine 5′-3′-O-(thio)triphosphate; G-protein, GTP-binding protein; GPAnt, GTP-binding protein antagonist; GP-2, zymogen granule glycoprotein-2; PIPES, 1,4-piperazinediethanesulfonic acid; CCK, cholecystokinin.
found that GPant-2a, but not GPant-1, enhanced calcium-stimulated amylase secretion from streptolysin-O permeabilized acini and that GPant-2a, but not GPant-1, inhibited GTPase activity on zymogen granules. These results suggest that Gq/11 on zymogen granules plays a tonic inhibitory role in calcium-regulated amylase release from pancreatic acini.

MATERIALS AND METHODS

Reagents—Streptolysin-O was purchased from Welcome Diagnostic (Greenville, NC), chromatographically purified collagenase from Worthington Biochemicals, bovine serum albumin (Fraction V), ICN Immunobiologicales (Lisle, IL), and protein A-agarose from Pierce. The following compounds were purchased from Sigma: MgATP, creatine kinase, creatine phosphate, GTP, and activated charcoal. \( \gamma^{\text{32P}} \text{GTP} \) (30 Ci/mmol) and \(^{125}\text{I}-\text{Bolton-Hunter CCK-8} \) (2200 Ci/mmol) were purchased from DuPont NEN (Boston, MA). GPant-1 and GPant-2A peptides were purchased from Sigma and Bio-Mol (Plymouth Meeting, PA), respectively. Affinity-purified polyclonal anti-\( \alpha_{q/11} \) antibody (C-19L), anti-\( \alpha \) antibody (K-20), anti-\( \alpha \) (C-10L), anti-G-protein-\( \beta \)-subunit antibody (T-20), and anti-phospholipase C\( \beta \) antibody (G-12) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-\( \alpha \) antisemur (RM-1) was purchased from DuPont NEN. Monoclonal antibody against rat pancreas GP2-2 was a gift from Prof. J. B. Lowe (Stanford University). Polyclonal antibody (G1B) to the a subunit of the sodium pump has been described previously (25). Secondary antibodies included peroxidase-coupled goat anti-rabbit immunoglobulin G (IgG, Amersham) and fluorescein isothiocyanate-labeled goat anti-rabbit IgG (Sigma).

Preparation of Pure Rat Acinar Zymogen Granules, Pure Zymogen Granule Membranes, and Plasma Membrane-Rich Fraction—Purified zymogen granules were prepared by Percoll gradient separation by previously published procedures (13, 26–28). Their purity has been determined both by analysis of several subcellular enzymatic organelle markers and by electron microscopic analysis and shown to be enriched 100–400-fold compared with other organelles (26). Granule membranes were isolated by ultracentrifugation after lysis with nigerin as described previously (29). Plasma membrane-rich fractions were prepared by sucrose gradient centrifugation by previously published procedures (29). \( ^{125}\text{I}-\text{CCK} \) binding to plasma membrane and zymogen granule membrane was carried out as described previously (29) except that the membranes were collected on Whatman GFF filters and washed three times with ice-cold buffer to terminate binding. Protein content of membranes was determined with the Bio-Rad protein assay kit, using bovine serum albumin as standard.

Western Blotting of Zymogen Granule Membranes and Plasma Membranes—Electrophoresis was performed as described (28). Variable amounts of protein from each sample were loaded per lane onto 7.5% or 10% SDS-polyacrylamide electrophoresis gels and run at 200 V. After gel electrophoresis, proteins were transferred to nitrocellulose membranes at 30 volts overnight. Western blotting was carried out as described previously, using the enhanced chemiluminescence reagent to visualize the secondary antibody.

Immunofluorescence Microscopy—Freshly prepared pancreatic lobules were fixed for 2 h at 4 °C with a mixture of 2% formaldehyde (prepared from paraformaldehyde) and 0.25% glutaraldehyde in phosphate-buffered saline. Fixed tissue was rinsed in phosphate-buffered saline, cryoprotected with sucrose, and frozen as described previously (13, 30). Immunofluorescence localization of \( \alpha_{q/11} \) in 5-\( \mu \)m thick cryostat sections followed the procedures described previously in detail (13, 31). Polyclonal anti-\( \alpha_{q/11} \) was used at 10 \( \mu \)g/ml. Specificity of staining was assessed by preincubation of primary antibody with 10-fold excess by weight of peptide used to generate the antibody. For comparative purpose, some cryostat sections were exposed to monoclonal antibody to the zymogen granule membrane marker, GP-2. Sections were examined by epifluorescence microscopy and confocal fluorescence microscopy (Bio-Rad MRC-600). Digitized images were processed using Photoshop 3.0 software.

Preparation of Pancreatic Acini and Amylase Release from Permeabilized Acini—Acini were prepared by collagenase digestion as described previously (32). After isolation, acini were suspended in incubation buffer consisting of 20 mM PIPES (pH 7.0), 140 mM potassium glutamate, 0.91 mM MgCl\(_2\), 5 mM EGTA, 1 mg/ml bovine serum albumin, 0.1 mg soybean trypsin inhibitor, 1 mM ATP (Mg salt), and 0.5 IU/ml streptolysin-O. For permeabilizing acini and introducing G-protein antagonist peptides into acini, aliquots of the acinar suspension were incubated at 37 °C for 1.5 min in the presence of various amounts of the indicated peptide, then placed in ice-cold water bath for 5 min and aliquoted into 200-\( \mu \)l samples. Amylase release was initiated by adding 200 \( \mu \)l of permeabilization buffer (0.025 M sucrose) to give a final concentration of 1 mM free Mg\(^{2+} \) and the specified concentrations of free Ca\(^{2+} \) which were calculated using a computer program as described previously (32). After incubation for 5 min at 30 °C, samples were centrifuged for 10 s in a microcentrifuge. Amylase released into the supernatant during the incubation was quantified using the Phadebas Amylase Test (Pharmacia, Columbus, OH) and expressed as a percent of total amylase in the acini at the beginning of the incubation.

Immunoprecipitation of \( \gamma^{\text{32P}} \)GTP-sequestered Protein from Purified Zymogen Granules—For immunoprecipitation of \( \alpha_{q/11} \), purified zymogen granules were sonicated for 15 s in lysis buffer containing 25 mM Tris (pH 7.5), 1.5 mM dithiothreitol, 3 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 2 mM EGTA, 10 \( \mu \)g/ml leupeptin, and 0.5% Triton X-100. The insoluble fraction was removed by centrifugation at 10,000 \( \times \) g for 10 min at 4 °C. The supernatant was incubated with 2 \( \mu \)g of \( \alpha_{q/11} \) antibody for 4 h followed by incubation with protein A-agarose beads for 1 h. The \( \alpha_{q/11} \)-bound beads were washed with the same buffer and used for Western blotting or assay of GTPase activity.

Measurement of GTPase Activity—Purified zymogen granules (200 \( \mu \)g of protein) or immunoprecipitated \( \alpha_{q/11} \) protein from purified zymogen granules (2 mg) were suspended in 60 \( \mu \)l of HEPES-buffered solution (50 mM HEPES, pH 7.2, 1 mM dithiothreitol). The reaction was initiated by adding 40 \( \mu \)l containing of 50 mM HEPES (pH 7.2), 1 mM dithiothreitol, 2.5 mM EGTA, 250 mM NaCl, 0.5 mg/ml creatine kinase, 5.5 mM creatine phosphate, 2.5 mM ATP, 2.5 \( \mu \)M GTP, and 3 \( \mu \)Ci of \( \gamma^{\text{32P}} \)GTP followed by incubation for 15 min at 30 °C. The reaction was terminated by the addition of 900 \( \mu \)l of cold phosphoric acid (pH 2.3) supplemented with 25% activated charcoal by weight. The samples were centrifuged at 10,000 \( \times \) g for 15 min at 4 °C and the \( [\text{32P}] \)Pi in the supernatant (300 \( \mu \)l) was determined by liquid scintillation counting. Nonenzymatic hydrolysis of \( \gamma^{\text{32P}} \)GTP was subtracted from all data.

RESULTS

Screening of Heterotrimeric G-Proteins on Pancreatic Zymogen Granule Membranes—To determine the heterotrimeric G-protein \( \alpha \)-subunit(s) localized to zymogen granules, we performed Western blotting of purified zymogen granule membranes and a plasma membrane-rich fraction using antibodies specific to \( \alpha_{q/11} \), \( \alpha_s \), \( \alpha_i \), and \( \alpha \). Fig. 1 shows that \( \alpha_{q/11} \), \( \alpha_s \), and \( \alpha_i \) were all present in a plasma membrane-rich fraction; \( \alpha \) was only faintly visualized in plasma membranes and was therefore not studied further. Of the four G proteins, only \( \alpha_{q/11} \) was detected on zymogen granule membranes in Western blotting. In addition, the Na\(^+\) pump \( \alpha \) subunit was found on the plasma membrane but not granule membrane (Fig. 1). To quantitate the relative abundance of \( \alpha_{q/11} \) on plasma membrane and zymogen granule membranes we carried out a dilution series of plasma membranes from 30 to 0.01% and compared the immunoblot signal to 30 \( \mu \)g of granule membranes. In two separate preparations 30 \( \mu \)g of plasma membranes were comparable to 1 \( \mu \)g of plasma membranes. Based on the method of preparation and relative purity of granules (26) this is not likely due to contamination. To confirm this quantitatively, we
Fig. 2. Western blotting of G-protein β-subunits and phospholipase Cβ1 in zymogen granule membranes and plasma membrane-rich fraction. 30 μg of purified zymogen granule membranes (ZGM) and plasma membrane-rich fraction (PM) were separated on 10% (panel A) or 7.5% (panel B) SDS minigels and transferred to nitrocellulose membranes. Membranes were then probed with anti-G-protein β-subunit antibody (panel A; 0.2 μg/ml) or anti-phospholipase Cβ1 antibody (panel B; 0.2 μg/ml) followed by detection with peroxidase-coupled anti-rabbit immunoglobulin G (1:5000) and visualized by enhanced chemiluminescence (ECL). The molecular mass standards are indicated on the left.

evaluated CCK receptor binding as a well established pancreatic plasma membrane marker. Specific binding was observed on plasma membranes (total binding 20,173 ± 211 cpm, nonspecific 318 ± 29, mean ± S.E., n = 4). By contrast, no specific binding was present on zymogen granule membranes (total binding 306 ± 24 cpm, nonspecific 340 ± 42).

Recently, multiple heterotrimeric G-protein α-subunits were shown to be localized to the Golgi apparatus of pancreatic acinar cells using immunohistochemistry, but no β-subunits were detected in the Golgi apparatus (33). These investigators suggested that G-protein α-subunits are localized to the Golgi apparatus independent of β-subunits. To determine whether αq/11 exists alone or as part of a complete G-protein complex, zymogen granule membranes were probed with a β-subunit antibody directed against a common region in the COOH-terminal of all β-subunits. A 37-kDa band was found to be present in both zymogen granule membranes and plasma membrane-rich fraction (Fig. 2A). These data suggest that αq/11 protein on zymogen granules maintains an interaction with β-subunit as an intact heterotrimeric G-protein as is the case for the plasma membrane. We also carried out Western blotting using antiphospholipase Cβ1 antibody; phospholipase Cβ1 was detected in the plasma membrane-rich fraction, but not in purified zymogen granule membranes (Fig. 2B). These observations in total, confirm the purity of our zymogen granule and indicate that αq/11 in granule membrane preparations is not due to contamination with plasma membranes.

Immunofluorescence Localization of Gq/11 in Pancreatic Acini—To further address the localization of αq/11, we analyzed the distribution of αq/11 protein in cryostat sections of pancreatic lobules by immunofluorescence and confocal fluorescence microscopy. As shown in Fig. 3A, αq/11 staining was pronounced along the basolateral membranes of acinar cells, and more weakly localized to the granule region in the apical cytoplasm. Although not quantitative, the weaker immunofluorescence signal over the granule area compared with the plasma membrane is consistent with the similar pattern resolved in Western blots (Fig. 1). When anti-αq/11 antiserum was preincubated with the peptide used to generate the antibody, the specific staining associated with both the basolateral membrane and the granules was abolished (Fig. 3B). Immunofluorescence staining of the apical cytoplasm with anti-αq/11 appeared at higher magnification to be punctate and is restricted to the basolateral membranes (arrowheads) and apical zymogen granules. The apical membrane bordering the acinar lumen (arrow) is unstained. Calibration bar, 10 μm.

Effect of G-protein Antagonist Peptides on Calcium-stimulated Amylase Secretion from Permeabilized Acini—To study the function of Gq/11 on zymogen granule membranes, we utilized the SP-related peptides GPant-1 and GPant-2a which are known to be antagonists of Gi/o and Gq/11, respectively (23). When these peptides are introduced into permeabilized acini and amylase secretion is stimulated by 10 μM free calcium, an effect on heterotrimeric G-proteins at the plasma membrane to mobilize intracellular calcium can be excluded and the function

Fig. 3. Immunofluorescence localization of αq/11 in pancreatic lobules by confocal fluorescence microscopy. As shown in A, staining for αq/11 is intense along basolateral membranes of acinar cells (arrowheads) and more weakly distributed to the granule region (arrows) in the apical cytoplasm. Little or no staining was present in the basal cytoplasmic regions of acinar cells, or in nuclei. As shown in B, specific staining of basolateral membranes and granules is abolished when αq/11 antibody was preincubated with competing peptide. A poorly defined, low level of fluorescence remained in the competed tissue sections. Photographic exposures and processing of images were identical for A and B. The localization of αq/11 is shown at higher magnification in a single acinus in C. Staining is punctate and is restricted to the basolateral membranes (arrowheads) and apical zymogen granules. The apical membrane bordering the acinar lumen (arrow) is unstained. Calibration bar, 10 μm.
of the G-proteins at steps distal to intracellular calcium mobilization can be evaluated. GPAnt-2a peptide potentiated amylase secretion induced by 10 μM free calcium from streptolysin-O permeabilized acini in a dose-dependent manner (Fig. 4). In contrast, GPAnt-1 did not alter calcium-stimulated amylase secretion. We also evaluated the peptide from the carboxyterminal of αq/11 used to generate the antibody and it had no effect on secretion (not shown). None of the peptides affected basal amylase secretion even at 100 μM. These data demonstrated that GPAnt-2a peptide specifically potentiated calcium-stimulated amylase release at a late step of exocytosis.

**Effect of G-protein Antagonist Peptides on GTPase Activity on Zymogen Granules**—To confirm that GPAnt-2a peptide augments calcium-stimulated amylase secretion from permeabilized acini by antagonizing G-proteins on zymogen granules, we evaluated the GTPase activity of zymogen granules. As shown in Fig. 5A, 100 μM GPAnt-2a peptide inhibited GTPase activity on zymogen granules approximately 25%. GPAnt-1 peptide, however, had no effect on the GTPase activity on zymogen granules. Since multiple small G-proteins are also known to be localized on zymogen granules (27, 34), total GTPase activity of zymogen granules most likely represents the activity of multiple G-proteins. Therefore, the fact that GPAnt2a inhibited only 25% of the GTPase activity on zymogen granules is not surprising.

To further determine that GPAnt-2a peptide specifically inhibits Gq/11 protein function on zymogen granules, we examined the inhibitory effect of G-protein antagonist peptides on GTPase activity of Gq/11 immunoprecipitated from purified zymogen granules. Fig. 5B shows immunoprecipitated Gq/11 protein from zymogen granules visualized by Western blotting. Using this immunoprecipitant, the effect of G-protein antagonist peptides on Gq/11 GTPase activity was assayed. As shown in Fig. 5C, GPAnt-2a peptide markedly inhibited immunoprecipitated Gq/11 GTPase activity by about 60%. GPAnt-1 peptide, however, had no effect on GTPase activity of Gq/11. Taken together, these data suggest that the inhibition of GTPase activity of zymogen granules by GPAnt-2A peptide results in the potentiation of calcium-stimulated amylase secretion from permeabilized acini.

**DISCUSSION**

Transduction and amplification of receptor-mediated signals to phospholipase C at the level of the plasma membrane is the only currently established function of Gq/11 (35, 36). Although some studies have predicted the participation of Gq/11 in intracellular vesicle trafficking (20, 37), such a role has not been conclusively demonstrated. In the present study, we have described a role for Gq/11 on secretory vesicles in exocytosis. We showed the localization of Gq/11 on zymogen granules by Western blotting, immunoprecipitation, and immunohistochemistry. We next demonstrated that Gq/11 antagonist peptide GPAnt-2a augmented calcium-stimulated amylase release from permeabilized acini. Furthermore, we observed that GPAnt-2a inhibited GTPase activity of both zymogen granules and immunopurified Gq/11 from zymogen granules. Accordingly, it is suggested that GPAnt-2a enhanced calcium-induced...
amylose secretion from permeabilized acini by inhibiting GTPase activity of G_{q/11} on zymogen granules.

Of central importance to our evaluation of the role of α_{q/11} on zymogen granule membranes is the purity of the granules and the certainty that the observed signal is not due to contamination with G_{q/11}-rich plasma membrane. The preparation of granules makes use of the high density of intact granules which band in Percoll gradients separate from other organelles and are enriched 250–500-fold relative to DNA (nuclei), glutamate dehydrogenase (mitochondria), and NADH dehydrogenase (endoplasmic reticulum) (26). In a recent study the purified granule preparation was used to show the absence of inositol 1,4,5-trisphosphate receptors or inositol 1,4,5-trisphosphate-induced Ca^{2+} release in purified granules (38) although both are present in contaminating membranes present in crude granules prepared by simple differential centrifugation. Finally, in the present work α_{q}, α_{s}, and α_{a} subunits of the sodium pump, phospholipase Cβ1, and CCKA receptors were all identified in the plasma membrane but not zymogen granule membranes; if the granule membranes were contaminated with as little as 1% of plasma membranes we would have detected saturable CCK binding in the granule membranes. The results with CCK binding are especially useful since there is a large and quantifiable signal in plasma membranes. Finally, the immunohistochemistry reveals specific labeling of the zymogen granules that could be completed by preincubating antisera with the immunizing peptide. Thus, the data as a whole makes a convincing case that α_{q/11} is present on the zymogen granules.

G-proteins have been suggested to be involved in a late step of exocytosis as a result of observations obtained using permeabilized cell systems. When a non-hydrolyzable GTP analogue, GTPγS was introduced into neutrophils (1), mast cells (2), insulin secreting RIN cells (3), and chromaffin cells (39), in the presence of free calcium, exocytosis was markedly enhanced. These findings strongly supported the involvement of G-proteins in the late steps of exocytosis. However, since GTPγS can activate both heterotrimeric G-proteins and small G-proteins, other reagents specific to trimeric G-proteins are required to analyze their function in exocytosis. AlF4 and mastoparan, both of which are known to be specific activators of heterotrimeric G-proteins, have been intensively used in permeabilized cell systems. When AlF4 was introduced into permeabilized secretory cells, their calcium-stimulated exocytosis was enhanced (40, 41) or attenuated (17). These findings strengthen the idea that heterotrimeric G-proteins are involved in the late steps of exocytosis. Although AlF4 is a powerful tool for studying heterotrimeric G-proteins, it activates all isoforms. By contrast, mastoparan, an amphiphilic tetradecapeptide purified from wasp venom, is more specific to inhibitory heterotrimeric G-proteins G_{i} and G_{o} (42, 43). Application of mastoparan to permeabilized cell systems suggest the direct linkage of G_{i} or G_{o} to the exocytotic machinery (44–46). In addition to mastoparan, pertussis toxin is also a specific tool to analyze G_{i} and G_{o}. It catalyzes their ADP-ribosylation, and results in their non-responsiveness to activating signals. Thus pertussis toxin pretreatment affected the exocytosis induced by exogenously introduced free calcium into permeabilized chromaffin cells (47, 48). Therefore, the participation of mastoparan and pertussis toxin sensitive G-proteins, G_{i} and G_{o}, in the late steps of exocytosis have been intensively studied in these cells. G_{i} and G_{o} were recently found on small synaptic vesicles of neuronal cells by immunocytochemistry, and G_{o} has also been found on large dense core vesicles of adrenal chromaffin cells (49). In exocrine tissues, both G_{i} and G_{o} have been reported on parotid secretory granule membranes by Western blotting (50). Since various proteins on secretory vesicles play important roles at the step of vesicle docking and fusion with plasma membrane (51, 52), heterotrimeric G-proteins on secretory vesicles are now assumed to be one of the G-proteins involved in the late steps of exocytosis. Recently, Vitale et al. (17, 18) have shown that G_{i} on secretory granules has an inhibitory effect on the final stages of exocytosis in chromaffin cells. In insulin-secreting β-cells, Konrad et al. (19) have described that G_{i} on insulin containing vesicles plays a stimulatory role in the mastoparan-induced insulin secretion. These data suggest that different heterotrimeric G-proteins on secretory granules play distinct roles in regulated exocytosis. Concerning other heterotrimeric G-proteins, G_{o} is shown to be localized to secretory granule membranes of neuroendocrine tissues and parotid glands (20, 48). However, little is known about its function.

G_{q/11} has not previously been reported on secretory granules although it was shown to exist in the Golgi apparatus in some neuroendocrine cells (20) and pancreatic acini (33). Its function in the Golgi is still unclear. In the current study, we have shown that G_{q/11} is present on pancreatic zymogen granules and acts to inhibit calcium-triggered exocytosis. These findings are consistent with the previous evidence that G-proteins interact with intracellular calcium to bring about exocytosis (2, 53). Moreover, our findings that G_{q/11} antagonist GPAnt-2a potently augmented calcium-induced amylase secretion, but not basal secretion, reinforces the synergistic role of G_{q/11} with intracellular free calcium in regulated exocytosis.

Currently, the process of exocytosis is thought to occur in two phases. The first step of docking and fusion of primed secretory vesicles to plasma membranes is completed in the first 5 min. The second step is the sequential release of vesicles from a reserve pool (54). According to this theory, our findings that GPAnt-2a potentely enhanced the initial amylase release over a 5-min incubation suggests that the target protein of GPAnt-2a, G_{q/11}, acts on the docking and fusion steps of exocytosis in acinar cells. Taken together, it is reasonable to speculate that the G_{q/11} target of GPAnt-2a is on the zymogen granules. To confirm our hypothesis, we examined the GPAnt-2a effect on both zymogen granules and G_{q/11} isolated from zymogen granules. GPAnt-2a inhibited both GTPase activities. Thus we have concluded that G_{q/11} on zymogen granules tonically inhibits calcium-induced amylase release from pancreatic acini.

Heterotrimeric G-proteins exist on plasma membranes as a complex of α, β, and γ subunits at resting state, and separates into free α subunit and a βγ complex when activated; βγ subunits are assumed always to exist as a complex (55, 56). We have shown that βγ-subunit as well as α_{q/11} is localized to zymogen granule membranes. Although we did not evaluate the presence of γ subunit, it is reasonable to conclude that G_{q/11} exists as a complete complex of all three subunits on zymogen granules.

In the classical signal transduction pathway involving G_{q/11} on the plasma membrane, phospholipase C is the sole effector protein known thus far. In our Western blotting studies, we could detect phospholipase Cβ1 in the plasma membrane-rich fraction but not in zymogen granule membranes (Fig. 2B). Although this observation reinforces the purity of the zymogen granule membrane preparation, it concomitantly implies that the effector of the G_{q/11} on zymogen granules still remains uncertain. F-actin has been assumed to play inhibitory roles in exocytosis by blocking secretory vesicle movement toward the plasma membrane (57). Vitale et al. speculated that the G-protein which is involved in the negative control of exocytosis may be coupled with some component controlling the subplasmalemmal cytoskeleton and the movement of secretory granules to the exocytotic sites (17). Their speculation is based on results concerning G_{o} of chromaffin secretory granules. How-
ever, the interaction between Gq and F-actin is not yet elucidated. On the other hand, Ibarrondo et al. (37) have recently reported the close association of Gq/11 with F-actin filaments in mammary tumor cell line. Moreover, it was shown that assembly and disassembly of F-actin are essential to the final steps of exocytosis (54). Taken together, it is more likely for Gq/11 that some protein(s) which regulates the actin network organization might be the effector of the G-proteins on secretory granules. Alternatively, the possibility still remains that undefined isoforms of phospholipase C might exist on zymogen granules as an effector of Gq/11. In any case, further studies are needed to elucidate the effector of Gq/11 on zymogen granules.

In conclusion, we have demonstrated a tonic inhibitory action of Gq/11 which localizes to zymogen granules in pancreatic acinar exocytosis. These observations provide new insights for understanding the synergistic regulation of exocytosis by intracellular calcium and G-proteins.

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