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$G\alpha_{i3}$ in Pancreatic Zymogen Granules Participates in Vesicular Fusion[1]

Akm A. Sattar,[*] Ramesh Boinpally,[*,†] Marvin H. Stromer,[‡] and Bhanu P. Jena[*,†,2]

Departments of [*]Physiology and [†]Pharmacology, Wayne State University School of Medicine, 540 East Canfield Avenue, Detroit, Michigan 48201; and [‡]Department of Animal Science and Biochemistry, Iowa State University, Ames, IA 50010

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Earlier studies indicated that a G_i-like protein localized in pancreatic zymogen granule (ZG) membrane mediates vesicle swelling, and is a potentially important prerequisite for vesicle fusion at the cell plasma membrane (PM) [Jena *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94, 13317–13322]. In the present study, we demonstrate the presence of $G\alpha_{i3}$ immunoreactivity in ZGs of rat exocrine pancreas using immunoblot assays, light and electron immunomicroscopy. Since GTP has been implicated in the fusion of isolated ZG with PM fractions [Nadin *et al.* (1989) *J. Cell Biol.* 109, 2801–2808], the potential role of ZG-associated $G\alpha_{i3}$ was investigated. Immunoblot assays demonstrate an increase in $G\alpha_{i3}$ protein in ZGs isolated from carbamylcholine stimulated pancreas. Thin layer chromatography shows an increase in GTP hydrolysis by GTPase in ZGs isolated from stimulated compared to resting pancreas. *In vitro* fusion assays demonstrate that ZGs isolated from carbamylcholine-stimulated pancreatic lobules fuse with the PM at a greater potency in the presence of GTP, mastoparan (G protein agonist) and its analogue mas7. Furthermore, $G\alpha_{i3}$-specific a recombinant GAIP (G alpha interacting protein), potentiates ZG-PM fusion in the presence of GTP but not in presence of the non-hydrolyzable GTP analogue Gpp(NH)p. Our immunoblot analysis demonstrates the recruitment of $G\alpha_{i3}$ immunoreactivity to ZG from stimulated acinar cells, and these isolated ZGs are more potent and efficient in fusing with plasma membrane fractions, suggesting the possible involvement of $G\alpha_{i3}$ in ZG-PM fusion. The participation of ZG-associated $G\alpha_{i3}$ in ZG-PM fusion is further confirmed by the influence of the $G\alpha_{i3}$-specific GAIP, which is known to interact specifically with $G\alpha_{i3}$, and not with $G\alpha_{i2}$ or $G\alpha_q$ [DeVries *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92, 11916–11920]. Additionally, our data suggest that GTP hydrolysis is a requirement for ZG-PM fusion since GAIP in the presence of Gpp(NH)p shows little or no effect on fusion, whereas GAIP in the presence of GTP significantly potentiates ZG-PM fusion. Our studies suggest a possible role for ZG-associated $G\alpha_{i3}$ in ZG-PM fusion.

Key words: heterotrimeric G protein, membrane fusion, pancreas, plasma membrane, zymogen granule.

GTP-binding proteins play a major role in the control of intracellular membrane trafficking (*1*). Most attention has focused on the low molecular weight GTP- binding proteins of the rab family. These proteins are widely distributed within cells and each organelle is enriched in particular members of the family (*2*). More recently, however, it has become increasingly clear that heterotrimeric G proteins are also involved in the control of membrane trafficking along the secretory pathway (*3–5*). For example, heterotrimeric G proteins play a role in vesicle budding from the *trans*-Golgi network and in regulated exocytosis in both chromaffin cells and mast cells (*6*). In exocrine pancreas, GTPγS has been shown to stimulate exocytosis in permeabilized acini and fusion between zymogen granules (ZGs), the membrane-bound secretory vesicles in exocrine pancreas, and PM preparations (*3, 7–8*). Furthermore, both monomeric and heterotrimeric G proteins are present in ZG membranes. Heterotrimeric G proteins play important roles in transmembrane signaling, as they participate in the processing and sorting of incoming signals as well as adjusting the sensivity of signaling system (*9*). Heterotrimeric G proteins are composed of Gα and Gβγ subunits. Agonist-liganded receptors activate Gα by inducing a change in conformation that leads to GDP release and GTP-binding. Gα-GTP dissociates from Gβγ, and both subunits can then interact with a variety of intracellular effectors (*10*). Gα and Gβγ remain activated until the intrinsic GTPase activity of Gα hydrolyzes GTP to GDP. The mechanism utilized by cells to respond to this signal is not well understood. However, recently, it has been shown that G protein Gβγ regulates exocytotic fusion by binding SNARE protein syn-

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[2] To whom correspondence should be addressed. Tel: +1-313-577-1532, Fax: +1-313-993-4177, E-mail: bjena@med.wayne.edu

Abbreviations: ZG, zymogen granule; PM, plasma membrane; GTP, guanosine 5′-triphosphate; Gpp(NH)p, guanosine 5′-[β,γ-imido]triphosphate; GDP, guanosine 5′-diphosphate; GMP, guanosine 5′-monophosphate; Gα, G protein alpha subunit; Gβγ, G protein beta and gamma subunits heterodimer; NaF, sodium fluoride; Mas, mastoparan; Mas7, mastoparan 7; GAIP, G alpha interacting protein.

taxin (*11*), SNAP-25, and the ternary SNARE complex, suggesting that Gβγ could directly target the fusion machinery to inhibit vesicular release (*12*). Many of these GTP-binding regulatory proteins belong to the Gi subfamily. It has been previously reported that a $G\alpha_i$-like protein present on the ZG membrane contributes to ZG swelling (*13*). We report here that $G\alpha_{i3}$ in pancreatic ZG participates in membrane fusion, and that the hydrolysis of GTP bound to $G\alpha_{i3}$ is a requirement for this process.

EXPERIMENTAL PROCEDURES

Isolation of Pancreatic Plasma Membrane—Rat pancreatic plasma membrane (PM) fractions were prepared by a modification of the method of Rosenzweig *et al.* (*14*). Male Sprague-Dawley rats weighing 70–100 g were euthanized by ether inhalation. Pancreata were removed and placed in ice-cold phosphate-buffered saline (PBS), pH 7.5. Adipose tissue was removed and the pancreatic tissue was diced into 0.5 mm^3 pieces using a razor blade in a few drops of homogenization buffer A (1.25 M sucrose, 0.01% trypsin inhibitor, and 25 mM HEPES, pH 6.5). The chopped tissue was homogenized in 15% (w/v) ice-cold homogenization buffer A using four strokes at maximum speed of a motor-driven pestle (Wheaton overhead stirrer). One-and-a-half milliliter of the homogenate was layered over a 125 μl cushion of 2 M sucrose and 500 μl of 0.3 M sucrose was layered onto the homogenate in Beckman centrifuge tubes. After centrifugation at 145,000 ×*g* for 1 h in a Sorvall AH-650 rotor, the materials banding between the 1.2 and 0.3 M sucrose interface were collected and the protein concentration was estimated. The sample was divided into aliquots and stored at –20°C. For each experiment an aliquot of PM was thawed on ice and used in ZG-PM fusion experiments.

Carbamylcholine Stimulation of Pancreatic Lobules—Pancreatic lobules were prepared according to a published method (2). The lobules were then incubated in a conical flask containing 4 ml of oxygenated lobule incubation buffer L (20 mM glucose, 120 mM NaCl, 4.8 mM KCl, 12 mM $MgCl_2$, 2 mM $CaCl_2$, 0.01% soybean trypsin inhibitor, 0.1% BSA, and 25 mM HEPES, pH 7.4) in the absence or presence of 10 μM carbamylcholine for 1 h in a shaking water bath at 37°C.

Electron Microscopy—Isolated pancreatic lobules were fixed in 125 mM MES buffer, pH 6.5, containing 4% paraformaldehyde for 2 h. Following fixation, the pancreatic lobules were washed with PBS. The lobules were then dehydrated through a series of 30, 50, 70, and 90% methanol. The lobules were embedded in unicryl and sectioned; the sections were transferred to coated specimen grids, incubated with anti $G\alpha_{i3}$ antibody, and then exposed to goat anti-rabbit IgG conjugated 5 nm gold particles. The immunostained sections on EM grids were then dried in the presence of uranyl acetate and methyl cellulose and examined in a transmission electron microscope.

Confocal Microscopy—Both the resting and carbamylcholine stimulated pancreatic lobules were fixed in 125 mM MES buffer, pH 6.5, containing 4% paraformaldehyde for 2 h. After washing with PBS, the lobules were incubated in 0.3 M sucrose for 3 h and then in 0.6 M sucrose overnight at 4°C. The tissues were infiltrated for 30 min with 2.1 M sucrose containing 20% poly-(vinyl)pyrolidine, mounted on holders, and rapidly frozen in liquid N_2. Cryosections (5 μm) were obtained with cryomicrotome, and the sections were placed on gelatin-coated glass slides. The sections were washed with 120 mM phosphate buffer, pH 7.4, for 30 min, and then blocked with the same buffer containing 0.5% goat serum and 0.7% Triton X-100. Then the sections were incubated with diluted $G\alpha_{i3}$ antibody for 1 h and washed three times with PBS for 10 min. The sections were incubated with diluted TRITC-conjugated donkey anti–rabbit IgG for 30 min, washed three times with PBS, and mounted in PBS containing 70% glycerol. The preparations were examined with a Carl Zeiss confocal microscope.

Isolation of Zymogen Granules—ZGs were purified by a modification of our published procedure (*15*). Rat pancreas or pancreatic lobules were chopped into 0.5 mm^3 pieces and suspended in 15% (w/v) ice-cold homogenization buffer B (0.3 M sucrose, 0.01% soybean trypsin inhibitor, and 25 mM HEPES, pH 6.5). The suspension was homogenized in a Teflon-glass homogenizer using two strokes at maximum speed of a motor-driven pestle (Wheaton overhead stirrer). The homogenate was then centrifuged for 5 min at 300 ×*g* at 4°C in a Sorvall SS34 rotor to obtain a supernatant. One volume of the supernatant fraction was mixed with two volumes of Percoll-Sucrose-HEPES buffer C (0.3 M sucrose, 86% percoll, 0.01% soybean trypsin inhibitor, and 25 mM HEPES, pH 6.5) and the mixture was centrifuged for 30 min at 16,000 ×*g* at 4°C in a Sorvall SS34 rotor. ZGs were obtained as a loose white pellet at the bottom of the tube. The ZGs were suspended in 100 mM MES buffer, pH 6.5, and the protein concentration of the suspension was assayed by the Bradford method (*16*). The volume of the ZGs preparation was adjusted to obtain a protein concentration of 200–400 μg/ml.

SDS-PAGE and Immunoblot Analysis—ZG proteins were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane for immunoblotting. The membrane was blocked with 5% skim milk in PBS for 1 h, and subsequently incubated with diluted $G\alpha_{i3}$ antibody in PBS–0.1%Tween-20 (1:1,000) for 1 h. After 3 washes for 10 min each in PBS–0.1% Tween-20, the membranes were incubated with diluted peroxidase-conjugated donkey anti-rabbit antibody for 30 min. The nitrocellulose membrane was washed 3 times in PBS-Tween (5 min each wash), treated with enhanced chemiluminescence solution, exposed to X-ray film, and developed.

GTPase Assays—To determine the GTPase activity of ZGs, isolated ZGs were incubated with [α-^{32}P]-GTP in assay buffer G (1 mM DTT, 1 mM EGTA, 20 mM $MgCl_2$, 0.2% BSA, and 25 mM HEPES, pH 6.5) at 37°C, and aliquots of the supernatant were withdrawn at different time points. The reaction was stopped immediately by adding an equal volume of 2% SDS–40 mM EDTA solution. A 3 μl aliquot of this reaction mixture was spotted on a PEI plate for analysis by a thin layer chromatographic method (*17*). The plates were developed in 1 M LiCl and autoradiographed.

Enzymatic ZG-PM Fusion Assay—Ten microliters of ZGs and 10 μl PM were incubated in the presence or absence of other reagents in an assay volume of 200 μl of 100 mM MES, pH 6.5, containing 25 mM KCl. The reaction was carried out at 37°C for 10 min in a shaking water bath. After incubation, the reaction tubes were placed in ice water to stop the reaction. Granules were pelleted by centrifugation for 2 min at 8,000 rpm in an Eppendorf centrifuge 5415C,

and half of the reaction volume (clear supernatant) was transferred to a separate Eppendorf tube. Both the supernatant and pellet portions were assayed for amylase following the method of Bernfield (*18*) using potato starch as the substrate. Briefly, 100 μl of samples appropriately diluted with amylase assay buffer D (10 mM NaH_2PO_4/10 mM Na_2HPO_4/20 mM NaCl) were mixed with the same volume of 1% starch solution in a borosilicate glass tube, and incubated for 10 min at 30°C in a shaking water bath. After cooling the tubes for 10 min in an ice-cooled water bath, 400 μl of coloring reagent E (44 mM dinitrosalicylic acid/200 mM KOH/20 mM Na-K tartrate) was added to the reaction mixture, and the tubes were boiled for 25 min. The tubes were cooled in an ice-cold water bath and 1.4 ml distilled water was added. Optical density was measured at 530 nm using a DU 530 life science uv/vis spectrophotometer. The results are expressed as a percentage of the total amylase. Each sample was assayed in triplicate. Values are means ± SEM.

Fluorimetric ZG-PM Fusion Assay—A ZGs suspension in sucrose/MES buffer, pH 6.5, protein concentration approximately 5 mg/ml, was labeled with a lipid-soluble fluorescent probe, octadecylrhodamine (R18), by incubation at 25°C for 3 min. Labeled ZGs were recovered by centrifugation at 8,000 rpm for 3 min in an Eeppendorf centrifuge 5415C and resuspended in the original volume of buffer. For ZG-PM fusion assay, ZGs were incubated with unlabeled PM in a temperature-regulated cuvette with constant stirring in a Hitachi F-2000 fluorescence spectrophotometer. Membrane fusion results in the redistribution of R18, which is detected as an increase in fluorescence (*8*). An excitation wavelength of 560 nm and emission at 590 nm was used to detect the R18 fluorescence intensity. Five microliters of labeled ZGs were incubated in 1 ml of 280 mM sucrose/5 mM MES buffer, pH 6.5, at 37°C until a steady baseline was obtained. Plasma membranes (5 μg protein) were then added, and the fluorescence signal was monitored for 200 s. Similarly, fluorescence was also monitored following the subsequent addition of any reagent to the mixture. For each experiment, the fluorescence signal was expressed as the percentage change from baseline.

RESULTS AND DISCUSSION

In the present study, we report that $G\alpha_{i3}$ in ZG participates in ZG-PM fusion. To determine the association of $G\alpha_{i3}$ with ZG, the subcellular distribution of $G\alpha_{i3}$ immunoreactivity was examined in pancreatic acinar cells by both electron microscopy (Fig. 1) and immunoblotting (Fig. 2A) quantified by densitometric scanning (Fig. 2B), as well as confocal microscopy (Fig. 3A). Our EM studies demonstrate the specific localization of $G\alpha_{i3}$ to ZG (Fig. 1). This finding is further supported by immunoblot assays (Fig. 2A) and earlier studies (*13*). To begin to understand the role of $G\alpha_{i3}$ in exocytosis in pancreatic acinar cells, the distribution of $G\alpha_{i3}$ immunoreactivity in resting and stimulated cells as well as the association of $G\alpha_{i3}$ in ZG obtained from resting and stimulated pancreas were examined. Our studies reveal that ZGs obtained from carbamylcholine-stimulated acini demonstrate relatively higher $G\alpha_{i3}$ immunoreactivity. How this translocation occurs is unknown at this time.

To determine further the association of $G\alpha_{i3}$ with ZG and its role in secretion, the subcellular distribution of $G\alpha_{i3}$ immunoreactivity was examined (Fig. 3A) in resting and stimulated pancreatic acinar cells by immunofluorescence confocal microscopy. In resting acini, although immunoreactivity was detectable throughout the cell, its primary localization was in the apical region. Pancreatic acinar cells are polarized secretory cells in which the ZGs are localized at the apical end. It is well established that following a secretory stimulus, there is release of granular contents at the apical lumen. In our study, stimulated cells demonstrate an enrichment in $G\alpha_{i3}$ immunoreactivity in the apical region as compared to resting cells. This suggests the involvement of $G\alpha_{i3}$ in exocytosis and prompted us to investigate its possible regulatory role in the fusion of isolated ZGs with PM preparations.

Since Gα is the only subunit known to possess GTPase activity, we set out to compare the GTPase activity of $G\alpha_{i3}$ in ZGs obtained from carbamylcholine-stimulated and resting pancreatic lobules. ZGs obtained from resting and carbamylcholine stimulated lobules were incubated for various periods in buffer containing [α-^{32}P]GTP. The reaction products were analyzed by thin layer chromatography (Fig. 3B). The differences in the time course paralleled the differences in GTP hydrolysis, i.e., GTP hydrolysis increased gradually as a function of time with the increased released of products (GDP and GMP). Stimulated (S) ZGs showed relatively higher GTPase activity compared to resting (R) ZGs. The increased GTPase activity (Fig. 3B) in ZGs from stimulated pancreas is consistent with their increased $G\alpha_{i3}$ content (Fig. 2A) as quantified by densitometric analysis (Fig. 2B).

To investigate the possible regulatory role of $G\alpha_{i3}$ protein in ZG-PM fusion, we carried out a series of in vitro experiments with isolated pancreatic ZGs and PM in the absence and presence of GTP. The percent fusion of ZG isolated from resting lobules increased in a GTP dose–dependent

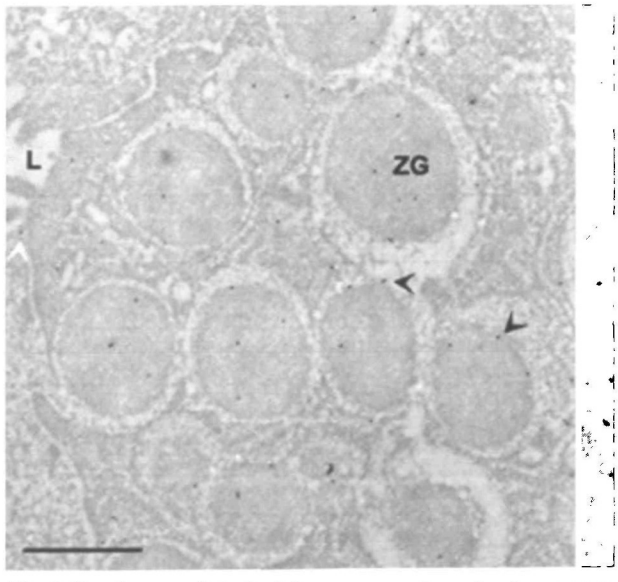


Fig. 1. **$G\alpha_{i3}$ is associated with zymogen granules in pancreatic acinar cells.** Pancreatic lobules were fixed, embedded, sectioned, and processed for electron microscopy. Electron micrographs of $G\alpha_{i3}$ immunogold-stained pancreatic tissue demonstrate the association of $G\alpha_{i3}$ with zymogen granules (ZG), present at the apical lumen (L). Arrowheads point to $G\alpha_{i3}$ immunogold localization. Bar = 1 μm.

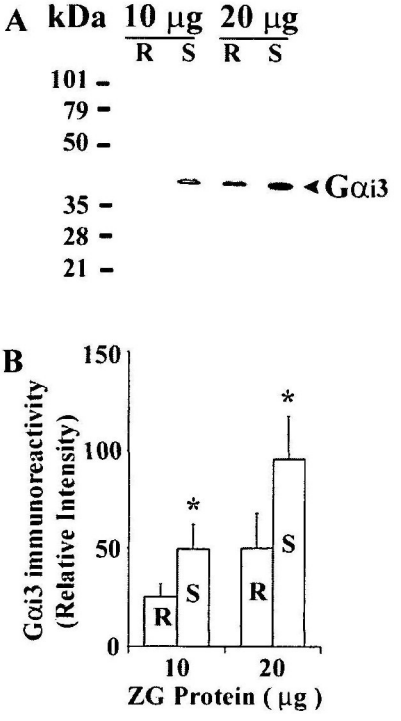


Fig. 2. **Enrichment of $G\alpha_{i3}$ in ZG following the stimulation of secretion.** A: $G\alpha_{i3}$ immunoreactivity in zymogen granules isolated from resting and carbamylcholine-stimulated pancreas. Ten and 20 μg of rat pancreatic zymogen granule proteins were resolved using 12.5% SDS-PAGE. The resolved proteins in each fraction were electrotransferred to nitrocellulose membranes and immunoblotted using a $G\alpha_{i3}$ specific antibody. A 42 kDa, $G\alpha_{i3}$-immunoreactive band was detected in resting (R) as well as in stimulated (S) zymogen granules. Note the intensity of $G\alpha_{i3}$ is much higher in ZG fractions obtained from stimulated acinar cells (S). The western blot shown is representative of three similar experiments. B: Immunoreactive $G\alpha_{i3}$ detected in the western blots was expressed and compared as relative band intensities of resting (R) *vs.* carbamylcholine-stimulated (S) zymogen granules. Values are the mean ± SEM of three separate western blot experiments, $*p < 0.05$ compared with resting. Densitometric analysis reveals a significant increase in $G\alpha_{i3}$ immunoreactivity in ZG obtained from stimulated acinar cells (S) compared to resting cells (R).

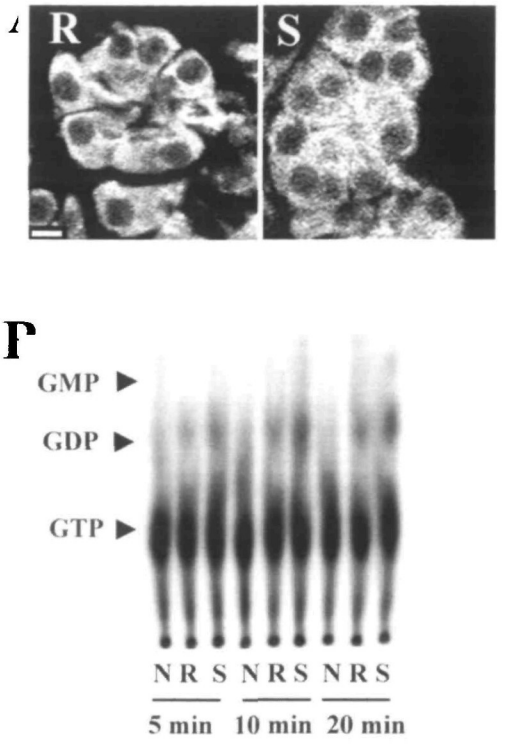
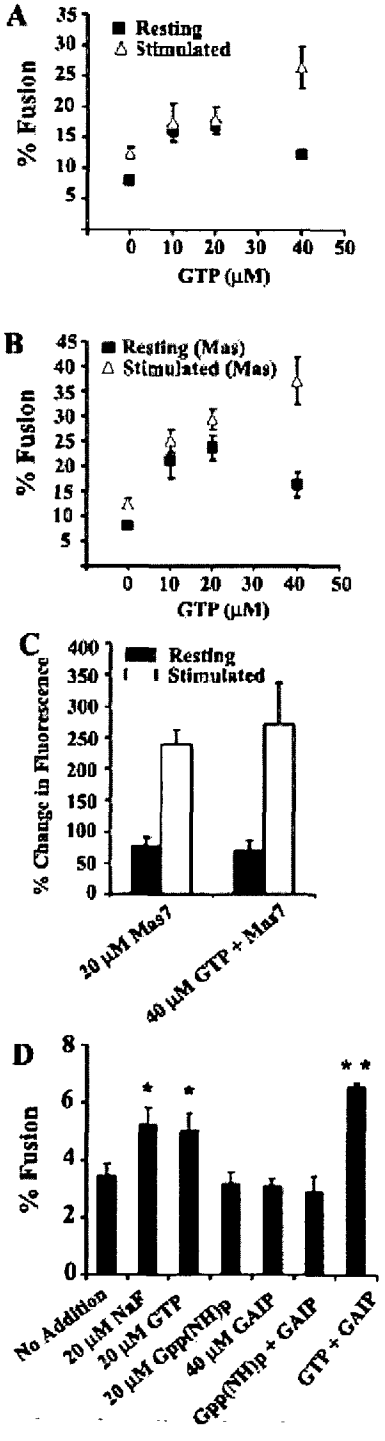


Fig. 3. **$G\alpha_{i3}$ in the secretion and hydrolysis of GTP.** A: $G\alpha_{i3}$ is associated with zymogen granules in pancreatic acinar cells, suggesting its involvement in secretion. Pancreatic lobules were incubated for 1 h at 37°C in the absence (R) or presence (S) of the secretorygogue carbamylcholine (1×10^{-6} M), and secretion was monitored. Following incubation, the lobules were fixed, cryosectioned and processed for confocal microscopy. Confocal images of $G\alpha_{i3}$ immunostained pancreatic tissue in the resting and stimulated states indicate the association of $G\alpha_{i3}$ with zymogen granules and its possible involvement in secretion. In resting acinar cells, $G\alpha_{i3}$ appears distributed throughout the cell, including in the apical region. The nucleus, located at the basolateral end of pancreatic acinar cells, is devoid of $G\alpha_{i3}$-specific immunostaining. Following the stimulation of secretion, much of the $G\alpha_{i3}$ immunoreactivity is found in the apical region of pancreatic acinar cells where secretion is known to occur. B: GTP hydrolysis by ZGs obtained from resting and stimulated pancreatic acinar cells. Autoradiograph of a thin layer chromatogram performed on [α-^{32}P]GTP (N) and [α-^{32}P]GTP in the presence of ZGs obtained from resting (R) and stimulated (S) acinar cells. Note a time dependent conversion of GTP to GDP and GMP by the stimulated ZGs as opposed to resting ZGs, a result also confirmed by densitometric scanning (data not shown).

manner (0–20 μM). At higher concentrations of GTP (40 μM), however, ZG-PM fusion showed little or no increase (Fig. 4A). Although, the percent fusion of ZG isolated from carbamylcholine-stimulated lobules did not show a dramatic increase at GTP doses up to 20 μM, there was a significant increase in ZG-PM fusion at 40 μM GTP (Fig. 4A). The reason ZG isolated from carbamylcholine-stimulated lobules did not show a significant upward trend in the enhancement of fusion at 10 or 20 μM GTP compared to resting ZG is not clear. One possible explanation may be the limiting amount of GTP under these assay conditions. In the presence of 40 μM GTP, however, ZGs isolated from carbamylcholine-stimulated lobules showed a significant increase in ZG-PM fusion compared with ZGs obtained from resting lobules. GTP dose–dependent ZG-PM fusion using ZGs isolated from resting and carbamylcholine-stimulated lobules was further examined in the presence of mastoparan (Fig. 4B). Mastoparan potentiated the fusion of ZGs obtained from both resting and carbamylcholine-stimulated lobules. However, GTP/mastoparan-induced ZG-PM fusion was further elevated using ZGs isolated from stimulated pancreatic lobules (Fig. 4B). Mastoparan, a tetradecapeptide that stimulates $G\alpha_{i3}$ at 20 μM, induced ZG-PM fusion in both the presence and absence of GTP (*19*). The effect of a mastoparan analogue, Mas7, on fusion was also examined by the fluorescent-dye method as described in "EXPERIMENTAL PROCEDURES." Mas7, in the absence or in presence of GTP also caused the potentiation of fusion of ZGs isolated from carbamylcholine-stimulated lobules as compared with resting lobules (Fig. 4C). These data further support the notion that $G\alpha_{i3}$ is enriched in ZG obtained from stimulated pancreas, resulting in increased hydrolysis of GTP and the potentiation of fusion with PM fractions.

To investigate whether GTP hydrolysis is a requirement

for ZG-PM fusion, we performed ZG-PM fusion assays in the presence of a non-hydrolysable-GTP analogue, Gpp(NH)p. Although, GTP-induced ZG-PM fusion is potentiated 15–35% over the control level (Fig. 4D), Gpp(NH)p had no effect on ZG-PM fusion (Fig. 4D), suggesting that GTP hydrolysis is a requirement for ZG-PM fusion. To confirm further the requirement of GTP hydrolysis for ZG-PM fusion, we assayed the effect on ZG-PM fusion of $G\alpha_{i3}$-specific GAIP, a recombinant protein that interacts specifically with $G\alpha_{i3}$ (*20*), in the absence or presence of GTP and Gpp(NH)p.



Although, GTP alone had a stimulatory effect on fusion, this effect was further extended in the presence of $G\alpha_{i3}$-specific GAIP (Fig. 4D). On the contrary, Gpp(NH)p had little or no effect on fusion in the presence of GAIP. GAIP alone also showed no effect on ZG-PM fusion (Fig. 4D). This stimulatory effect of GAIP in the presence of GTP and the absence of this stimulatory effect of GAIP in the presence of Gpp(NH)p on ZG-PM fusion further suggest that GTP hydrolysis is a requirement for ZG-PM fusion. To confirm further that the GTP effect is G-protein–mediated and not due to a ras-like small GTP-binding protein function, NaF was used in ZG-PM fusion assays. Sodium fluoride is a known activator of heterotrimeric G protein (*8*), and, like GTP, sodium fluoride stimulated ZG-PM fusion (Fig. 4D), confirming the involvement of G-protein in the process.

These studies demonstrate the selective localization of $G\alpha_{i3}$ in ZGs and its ability to hydrolyze GTP using its intrinsic GTPase activity *in vitro*. These studies further demonstrate that GTP, NaF, mastoparan, and its analogue, mastoparan 7, potentiate ZG-PM fusion. Additionally, we show here that the $G\alpha_{i3}$-specific GAIP, potentiates GTP-induced ZG-PM fusion. Together, these data demonstrate that $G\alpha_{i3}$ located at the membranes of secretory vesicles in pancreatic acinar cells, is involved in the fusion and/or expulsion of vesicular contents. Further studies, however, are necessary to determine the exact function of the ZG-associated $G\alpha_{i3}$-like GTP binding proteins in regulating vesicle–plasma membrane fusion. In addition, the possible involvement of other Gi-like proteins has to be examined (*3*). In agreement with our present findings, recent studies implicate G proteins in exocytosis (*11, 12*). The $G\beta\gamma$ subunits of G proteins regulate exocytotic fusion by binding SNARE proteins (*11*), and may directly target the fusion machinery to inhibit vesicular release (*12*). Our studies further demonstrate for the first time that ZG-associated $G\alpha_{i3}$ participates in membrane fusion in pancreatic acinar cells.

Fig. 4. **$G\alpha_{i3}$ participates in ZG-PM fusion.** A: As reported previously (*8*), ZG isolated from stimulated acinar cells demonstrate a GTP dose–dependent increase in ZG-PM fusion. Values are the mean ± SEM of four to six experiments at each GTP dose. B: A further potentiation of ZG-PM fusion was obtained in the presence of the $G\alpha_{i3}$ stimulant, mastoparan. Values are the mean ± SEM of six experiments at each GTP dose. C: The biochemical demonstrations of ZG-PM fusion in A and B were further confirmed by ZG-PM fluorimetric assays. Note an increase in fluorescence dequenching in ZG-PM fusion using ZG obtained from stimulated acinar cells (C). Values are the mean ± SEM of three experiments. D: The involvement of a heterotrimeric G-protein in ZG-PM fusion is further demonstrated in the presence of NaF. Values are the mean ± SEM of five experiments, $^*p < 0.05$ compared with no addition (control). Note the requirement of GTP hydrolysis in ZG-PM fusion. Values are the mean ± SEM of eight experiments, $^*p < 0.05$ compared with no addition (control). Gpp(NH)p, a non-hydrolyzable GTP analogue, has no effect on fusion. GAIP, a G alpha interacting protein, shows no effect on fusion either in absence or presence of Gpp(NH)p. GAIP potentiates ZG-PM fusion significantly in the presence of GTP. Values are the mean ± SEM of five experiments, $^{**}p < 0.005$ compared with no addition (control) and $^*p < 0.05$ compared with GTP induced fusion. The ability of GAIP to stimulate ZG-PM fusion in the presence of GTP and not in the presence of Gpp(NH)p further suggests that GTP hydrolysis is necessary for ZG-PM fusion.

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