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Islet Complex Lipids:

Involvement in the Actions of Group VIA Calcium-Independent Phospholipase A_2 in β-Cells

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Abstract

The β-isoform of group VIA calcium-independent phospholipase A_2 (iPLA$_2$β) does not require calcium for activation, is stimulated by ATP, and is sensitive to inhibition by a bromoenol lactone suicide substrate. Several potential functions have been proposed for iPLA$_2$β. Our studies indicate that iPLA$_2$β is expressed in β-cells and participates in glucose-stimulated insulin secretion but is not involved in membrane phospholipid remodeling. If iPLA$_2$β plays a signaling role in glucose-stimulated insulin secretion, then conditions that impair iPLA$_2$β functions might contribute to the diminished capacity of β-cells to secrete insulin in response to glucose, which is a prominent characteristic of type 2 diabetes. Our recent studies suggest that iPLA$_2$β might also participate in β-cell proliferation and apoptosis and that various phospholipid-derived mediators are involved in these processes. Detailed characterization of the iPLA$_2$β protein level reveals that β-cells express multiple isoforms of the enzyme, and our studies involve the hypothesis that different isoforms have different functions.

CLASSIFICATION OF PHOSPHOLIPASE A_2 AND FEATURES OF A GROUP VIA PHOSPHOLIPASE A_2 (iPLA$_2$β)

Phospholipase A_2 (PLA$_2$) (1) is a diverse group of enzymes that catalyze hydrolysis of the *sn*-2 substituent from glycerophospholipid substrates to yield a free fatty acid and a 2-lysophospholipid (1). At present, the recognized PLA$_2$s are classified into 14 different groups, based on their calcium requirement for activation and sequence homology (2). These include the low–molecular weight secretory PLA$_2$s (groups IB, IIA, IID, IIE, IIF, III, V, X, and XII) and the higher–molecular weight Ca^{2+}-dependent cytosolic PLA$_2$s (groups IVA, IVB, and IVC) and the Ca^{2+}-independent PLA$_2$s (groups VIA and VIB).

Among the PLA$_2$s is an 84-kDa (752 amino acid residues) cytosolic PLA$_2$ that does not require Ca^{2+} for catalysis. This PLA$_2$ has now been cloned from several sources (3–5), including rat and human pancreatic islet β-cells (4,6), is classified as group VIA PLA$_2$, and is designated the β-isoform of group VIA calcium-independent phospholipase A_2 (iPLA$_2$β) (7–9). Salient features (10,11) of the iPLA$_2$β amino acid sequence (Fig. 1) include eight NH_2-terminal ankyrin repeats, a caspase-3 cleavage site, an ATP-binding domain, a serine lipase consensus sequence (GXSXG), a bipartite nuclear localization sequence, and a

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COOH-terminal calmodulin-binding domain(s). An 88-kDa iPLA$_2$β isoform is also expressed in human pancreatic islets and is the product of an mRNA species that arises from alternate splicing (6). This isoform contains a 54–amino acid sequence that interrupts the eighth ankyrin repeat.

PROPOSED FUNCTIONS FOR iPLA$_2$β

Phospholipid remodeling

A housekeeping role involving generation of lysophospholipid acceptors for incorporation of arachidonic acid into phospholipids has been proposed for iPLA$_2$β, based on experiments involving inhibition of iPLA$_2$β activity in P388D1 cells with the bromoenol lactone (BEL) suicide substrate or with an antisense oligonucleotide (10,11). Inhibition of iPLA$_2$β activity in P388D1 cells suppressed (~60%) incorporation of [^{3}H]-arachidonic acid into phospholipids while reducing (~60%) [^{3}H]-lysophosphatidylcholine levels in [^{3}H]-choline–labeled P388D1 cells. However, [^{3}H]-palmitic acid incorporation was reduced only slightly. This is thought to represent the mechanism whereby iPLA$_2$β inhibition reduces incorporation of [^{3}H]-arachidonic acid into P388D1 cell phospholipids. Such incorporation reflects a deacylation/reacylation cycle (12) of phospholipid remodeling rather than de novo synthesis (13), and the level of lysophosphatidylcholine acceptors is thought to limit the rate of [^{3}H]-arachidonic acid incorporation into P388D1 cell phosphatidylcholine (10,11).

A second housekeeping function for iPLA$_2$β is suggested from studies with CTP:phosphocholine cytidyltransferase (CT)-overexpressing cells (14). CT catalyzes the rate-limiting step in phosphatidylcholine biosynthesis via the Kennedy pathway, and cells overexpressing CT exhibit increased rates of phosphatidylcholine biosynthesis and degradation and little net change in phosphatidylcholine accumulation (14). The increased phosphatidylcholine degradation observed in CT-overexpressing cells is prevented by BEL, and immunoreactive iPLA$_2$β protein and activity increase in such cells, suggesting that iPLA$_2$β is upregulated in response to CT overexpression (14). If general, this could represent an important role for iPLA$_2$β in cell biology because phosphatidylcholine biosynthesis is involved in regulation of the cell cycle and apoptosis (15).

Cell proliferation

Inhibition of iPLA$_2$β with BEL is reported to reduce arachidonic acid release, [^{3}H]-thymidine incorporation, and rates of lymphocyte (16) and Caco-2 (17) cell proliferation. In view of findings that arachidonic acid and/or its metabolites can stimulate *c*-fos, *c*-jun, or mitogen-activated protein kinase (18), it is possible that iPLA$_2$β might affect nuclear events involved in cell division.

Apoptosis

Involvement of iPLA$_2$β in processes leading to apoptotic cell death is suggested by several lines of evidence. Induction of apoptosis of U927 cells by anti-fas antibody is associated with hydrolysis of arachidonic acid from membrane phospholipids that is not catalyzed by group IV cytosolic phospholipase A$_2$, which is inactivated by caspases during apoptosis. The release of arachidonic acid is not inhibited by inhibitors of group II secretory phospholipase A$_2$, but the release is suppressed by the iPLA$_2$β inhibitor BEL (19). Both arachidonate 12-lipoxygenase and inducible nitric oxide synthase (iNOS) knockout mice are resistant to the diabetogenic effects of low doses of streptozotocin (20,21). Interleukin-1β–induced generation of nitric oxide in pancreatic islets causes accumulation of arachidonic acid and augments production of the arachidonic acid metabolite 12-hydroxy-(5,8,10,14)-eicosatetraenoic acid (12-HETE), and these effects are prevented by BEL (22). Induction of apoptosis in human promonocytic U937 cells is associated with activation of iPLA$_2$β after

proteolysis (23). Collectively, these observations suggest that iPLA$_2$β is involved in releasing arachidonic acid from membrane phospholipids in apoptosis and that arachidonic acid and/or its metabolites serve mediator functions in apoptosis.

Signal transduction

Studies in various cellular systems suggest that iPLA$_2$β might also participate in various signaling pathways. Evidence in support of this role include the observations that BEL, at concentrations that inhibit iPLA$_2$β, suppresses *1*) parathyroid-induced generation of arachidonic acid in rat proximal tubules (24), *2*) stimulated superoxide generation in neutrophils (25,26), *3*) interleukin-1β–stimulated increases in iNOS protein and nitric oxide generation in cardiac myocytes (27), *4*) cAMP response element binding protein phosphorylation during the period of myocardial ischemia (28), and *5*) virus-induced or double-stranded RNA-induced activation of iNOS expression by macrophages (29). Because pancreatic β-cells also express iPLA$_2$β, we have examined the potential role(s) of this group VIA Ca^{2+}-independent iPLA$_2$β in β-cells, and our findings are discussed below.

POTENTIAL ROLES OF iPLA$_2$β IN β-CELLS

Evidence for a role in signal transduction

Glucose and other fuel secretagogues induce hydrolysis of phospholipids in β-cell membranes, and this is reflected by accumulation of phospholipid-derived mediators including inositol 1,4,5-triphosphate, free arachidonic acid, and arachidonate metabolites (30). Metabolism of fuel secretagogues to yield ATP is an obligatory event in their induction of hydrolysis of arachidonate from β-cell membrane phospholipids (30,31) just as it is for insulin secretion (32). The fact that fuel secretagogue-induced hydrolysis of membrane phospholipids is, in part, independent of Ca^{2+} influx (33) suggests that a Ca^{2+}-independent phospholipase such as a iPLA$_2$β might be involved in this process.

Pancreatic islets, islet β-cells, and glucose-responsive insulinoma cells all express iPLA$_2$β mRNA and iPLA$_2$β enzymatic activity that is stimulated by ATP and inhibited by BEL (4,34–37). Inhibition of β-cell iPLA$_2$β activity with BEL suppresses glucose-stimulated hydrolysis of arachidonic acid from membrane phospholipids, the rise in cytosolic $[Ca^{2+}]_i$, and insulin secretion (34–37). However, BEL does not inhibit incorporation of labeled fatty acids into either pancreatic islet or insulinoma cell membrane phospholipids (9,38). Whereas BEL, at concentrations that inhibit iPLA$_2$β, does not inhibit group IV cytosolic PLA$_2$ (37) or glucose oxidation (35), which requires both glycolytic metabolism and mitochondrial oxidation of glucose, in pancreatic islets, it has been recognized to decrease the ATP/ADP ratio in mouse islets (39) and also inhibit phosphatidate phosphohydrolase (40). In view of such reports, nonpharmacological approaches involving molecular biological manipulations were used to explore involvement of the iPLA$_2$β protein in β-cell function. Attempts to suppress β-cell iPLA$_2$β activity with antisense oligonucleotides were ineffective (38); therefore, effects of an alternate approach of overexpressing iPLA$_2$β in insulinoma cells were examined.

INS-1 cells were stably transfected with a retroviral vector containing an iPLA$_2$β cDNA insert (9). Two stably transfected lines were isolated that overexpressed iPLA$_2$β activity and protein by 10-fold compared with the parental cell line. These were designated overexpressing (OE) cells. Insulin secretion and phospholipid remodeling in OE cells were then examined. Relative to the secretory responses observed with INS-1 cells transfected with empty retroviral vector that did not contain iPLA$_2$β cDNA, INS-1 cells that overexpressed iPLA$_2$β exhibited a much greater insulin secretory response to glucose alone and to glucose in combination with the cAMP-elevating agents forskolin or isobutylmethylxanthine (9,41). Pretreatment of INS-1 cells with BEL suppressed stimulated

insulin secretion, but neither overexpression of iPLA$_2$β nor inhibition of iPLA$_2$β enzymatic activity in the iPLA$_2$β-overexpressing INS-1 cells with BEL affected the rate or extent of arachidonic acid incorporation into INS-1 cell phosphatidylcholine.

Overexpression of iPLA$_2$β as a fusion protein with enhanced green fluorescence protein in INS-1 cells permitted examination of changes in its intracellular location after stimulation (Fig. 2), and glucose plus cAMP-elevating agents were found to induce accumulation of iPLA$_2$β in the perinuclear region. Such intracellular translocation appears to require cAMP-dependent protein kinase A–mediated phosphorylation events because inhibition of protein kinase A with H89 prevented stimulated perinuclear accumulation of iPLA$_2$β (41). Nuclear association of iPLA$_2$β induced by cAMP-elevating agents in INS-1 cells is of interest because glucose promotes both β-cell insulin secretion and proliferation, and glucose-induced INS-1 cell mitogenesis is cAMP-dependent (42). Because membranes of the nucleus and endoplasmic reticulum (ER) are contiguous (43), perinuclear accumulation of iPLA$_2$β is consistent with its association with a subcellular compartment that is likely to include the ER. Using organelle-specific trackers, association of iPLA$_2$β with β-cell ER and Golgi compartments upon stimulation has been observed (44). The likelihood that iPLA$_2$β might associate with the nucleus is also supported by the presence of a bipartite nuclear localization consensus sequence (^{511}KREFGEHTKMTDVKKPK^{527}) in the deduced amino acid sequence of iPLA$_2$β (45). Taken together, the above findings suggest that iPLA$_2$β has a signaling role in the β-cell, although iPLA$_2$β does not appear to participate in β-cell phospholipid remodeling or in phosphatidylcholine homeostasis.

iPLA$_2$β and β-cell proliferation

The iPLA$_2$β-overexpressing INS-1 cell line also proliferates more rapidly than nontransfected parental or empty-vector transfected INS-1 cells. This is reflected by a greater rate of increase in cell number in cultures of iPLA$_2$β-overexpressing INS-1 cells (46). The bases for these phenomena have not been determined, but the presence of a bipartite nuclear localization consensus sequence in iPLA$_2$β raises the possibility that iPLA$_2$β might affect nuclear events involved in cell division (18). Further, iPLA$_2$β activation converts phosphatidic acid to lysophosphatidic acid (LPA) (5), and LPA is a potent mitogen (16,47). Enhanced proliferation might result from a rise in cellular LPA levels that occurs as a consequence of iPLA$_2$β overexpression, and OE cells have been demonstrated to contain higher levels of lysophospholipid species than control cells.

iPLA$_2$β and ER stress–mediated β-cell apoptosis

Apoptosis is involved in β-cell death in type 1 diabetes (48) and might also contribute to β-cell death in type 2 diabetes (49). At present, three apoptotic signaling pathways are recognized (50). These are the extrinsic death receptor pathway involving adaptor molecules, the intrinsic mitochondrial pathway, and the ER stress pathway.

Agents that deplete ER Ca^{2+} stores, such as thapsigargin (51), induce apoptosis of MIN-6 insulinoma cells by a pathway that does not require increases in $[Ca^{2}]_i$ but that does require generation of the arachidonic acid metabolite 12-HETE (52). Thapsigargin has been demonstrated to induce hydrolysis of arachidonic acid from islet membrane phospholipids, and this is suppressed by the iPLA$_2$β inhibitor BEL (53). These findings suggest that ER stress induced by Ca^{2+} store depletion activates iPLA$_2$β, which then hydrolyzes membrane phospholipids to yield products that promote β-cell death.

In support of this hypothesis are the findings that thapsigargin induces a threefold increase in apoptosis of parental INS-1 cells (control, 4.2 ± 0.2% vs. plus thapsigargin, 12.3 ± 0.7%, $P < 0.05$), and apoptosis is suppressed by BEL. Whereas the spontaneous incidence of

apoptosis in control OE cells (3.7 ± 0.5%) is similar to that in parental cells, the apoptotic effect of thapsigargin is greatly amplified in the OE cells (Fig. 3*A*), and apoptosis is significantly suppressed by BEL. Thapsigargin also induces higher iPLA$_2$β activity and the generation of a 62-kDa iPLA$_2$β-immunoreactive protein (Fig. 3*B*). Inhibition of caspase-3 prevents both thapsigargin-induced apoptosis and perinu-clear accumulation of iPLA$_2$β (Fig. 3*C*). Immunoblotting and immunofluorescence analyses reveal that a 62-kDa iPLA$_2$β-immunoreactive protein accumulates in the perinuclear region of thapsigargin-treated INS-1 cells. These findings suggest that caspase-3 that is activated during apoptosis cleaves the 84-kDa iPLA$_2$β at its consensus sequence site to generate a 62-kDa product in INS-1 cells.

Caspase-3–catalyzed cleavage of iPLA$_2$β has been reported to occur in U937 promonocytes induced to undergo apoptosis, and the COOH-terminal product appears to be constitutively activated (23). Overexpression of the full-length iPLA$_2$β or the COOH-terminal caspase-3 proteolysis product of iPLA$_2$β (^{184}aa → COOH-terminal) in human embryonic kidney cells resulted in a higher incidence of apoptotic cell death and greater induction of arachidonic acid release from cells overexpressing the truncated iPLA$_2$β compared with those overexpressing full-length iPLA$_2$β. These findings suggest that the iPLA$_2$β product of a caspase-3–mediated cleavage was more active than the full-length iPLA$_2$β. It was proposed that the more active shorter iPLA$_2$β isoform could stimulate excessive turnover of nuclear membrane phospholipids, disrupt membrane fluidity, and promote apoptotic cell death.

Our findings suggest that induction of ER stress in INS-1 cells by depleting Ca^{2+} stores with thapsigargin stimulates iPLA$_2$β activity and promotes caspase-3–mediated cleavage of the INS-1 cell iPLA$_2$β. The resultant accumulation of a 62-kDa iPLA$_2$β-immunoreactive product in the perinuclear region of INS-1 cells raises the possibility that this might be one mechanism by which iPLA$_2$β participates in ER stress–induced apoptosis in β-cells.

Our studies also reveal that thapsigargin induces increased generation of ceramides in the OE cells compared with control cells. Ceramides (a family of 2-*N*-acylsphingosines) are important lipid second messengers that have been implicated as suppressors of cell growth and inducers of apoptosis (54). They can be generated by sphingomyelinase-catalyzed sphingomyelin hydrolysis (55), de novo synthesis (56), or as a consequence of decreased ceramidase activity (57), raising the possibility that any one of these ceramide-generating pathways might also be affected by iPLA$_2$β.

β-Cell iPLA$_2$β is a candidate for posttranslational modification

To examine the possibility that iPLA$_2$β undergoes posttranslational modifications that affect signaling, recombinant 84-kDa iPLA$_2$β was purified from sf9 cells after a sequential anion exchange and ATP affinity and calmodulin affinity chromatography protocol (58). NH_2-terminal amino acid sequencing analyses of the purified material yields a sequence that begins with residue 11 of the iPLA$_2$β-deduced amino acid sequence, reflecting loss of the first 10 amino acid residues. Liquid chromatography/electrospray ionization/mass spectrometry (Fig. 4*A*) analyses of a tryptic digest of the purified iPLA$_2$β reveal a peptide corresponding to residues 12–23 of the deduced amino acid sequence that would not be expected to arise from trypsin digestion of full-length iPLA$_2$β but that would be expected from an iPLA$_2$β variant lacking the first 11 amino acid residues. In the full-length iPLA$_2$β sequence, the first tryptic cleavage site occurs between residues 6 and 7 and the second between residues 23 and 24. Trypsin digestion thus yields peptides 1–6 and 7–23. In an iPLA$_2$β variant that lacks residues 1–11, trypsin digestion yields peptide 12–23. Peptide 12–23 is thus a signature peptide for this NH_2-terminally processed iPLA$_2$β variant. The presence of this peptide in the tryptic digest of purified iPLA$_2$β is reflected by the presence of $[M + H]^{+1}$ and $[M + 2H]^{+2}$ ions at *m/z* 1368.6 and 685.3, respectively. In addition, the expected aa$^{7–23}$ peptide from intact iPLA$_2$β is also present in the tryptic digest (Fig. 4*B*), as

is the $aa^{1–6}$ peptide modified at the NH_2-terminal by acetylation. This NH_2-blocked peptide would not yield NH_2-terminal sequence upon Edman analyses of the intact $iPLA_2\beta$ protein. Although the mechanisms responsible for generating an $iPLA_2\beta$ protein lacking the first 11 amino acids have not yet been determined, these findings indicate that the β-cell $iPLA_2\beta$ undergoes posttranslational modification by NH_2-terminal processing, and this might represent a means to regulate the activity, subcellular location, or protein–protein interactions of $iPLA_2\beta$.

Multiple isoforms of $iPLA_2\beta$

The $iPLA_2\beta$ cDNA first cloned from a rat pancreatic library encodes an 84-kDa protein, and human islets and lymphocytes were subsequently shown to express two $iPLA_2\beta$ mRNA species that arise by alternate splicing and encode 84- and 88-kDa proteins (6,59). Recent studies reveal predominant expression of an $iPLA_2\beta$-immunoreactive protein with an apparent molecular mass of 70 kDa in robustly glucose-responsive 832/13 INS-1 cells (60). These cells continue to express cytosolic $iPLA_2\beta$ activity that is stimulated by ATP and inhibited by BEL. Additionally, inhibition of 832/13 $iPLA_2\beta$ activity with BEL suppresses glucose-stimulated insulin secretion but does not affect arachidonate incorporation into phosphatidylcholine. Therefore, the BEL-sensitive catalytic activity expressed in 832/13 INS-1 cells is most likely attributable to a 70-kDa $iPLA_2\beta$-immunoreactive protein because it is essentially the only $iPLA_2\beta$-immunoreactive protein present in the cytosol of these cells.

CONCLUSIONS

A diminished capacity of β-cells to secrete insulin in response to glucose is a prominent characteristic of type 2 diabetes, and this motivates studies to achieve a fuller understanding of glucose-sensing mechanisms within the β-cell. Our findings suggest that $iPLA_2\beta$ participates in β-cell signal transduction. The expression level of $iPLA_2\beta$ appears to affect β-cell proliferation and apoptosis, and the enzyme may thus be an important participant in the life cycle of the β-cell. $iPLA_2\beta$ does not appear to participate in arachidonate acid incorporation into phospholipids or phosphatidylcholine homeostasis in β-cells, although such housekeeping functions of the enzyme have been proposed for other cells. The phospholipase activity of $iPLA_2\beta$ would result in the production of phospholipid-derived mediators including arachidonic acid and arachidonate metabolites, and lysophospholipids and the $iPLA_2\beta$ expression level also affect cellular content of the lipid second messenger ceramide. The finding that multiple $iPLA_2\beta$-immunoreactive isoforms are expressed in β-cells raises the possibility that different isoforms serve different functions that vary with the stimulation condition, subcellular compartment, phase of cell cycle, levels of interacting proteins, or other factors.

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Glossary

12-HETE	12-hydroxy-(5,8,10,14)-eicosatetraenoic acid
BEL	bromoenol lactone
CT, CTP	phosphocholine cytidyltransferase

ER	endoplasmic reticulum
iNOS	inducible nitric oxide synthase
iPLA$_2$β	β-isoform of group VIA calcium-independent phospholipase A$_2$
LPA	lysophosphatidic acid
OE	over-expressing
PLA$_2$	phospholipase A$_2$

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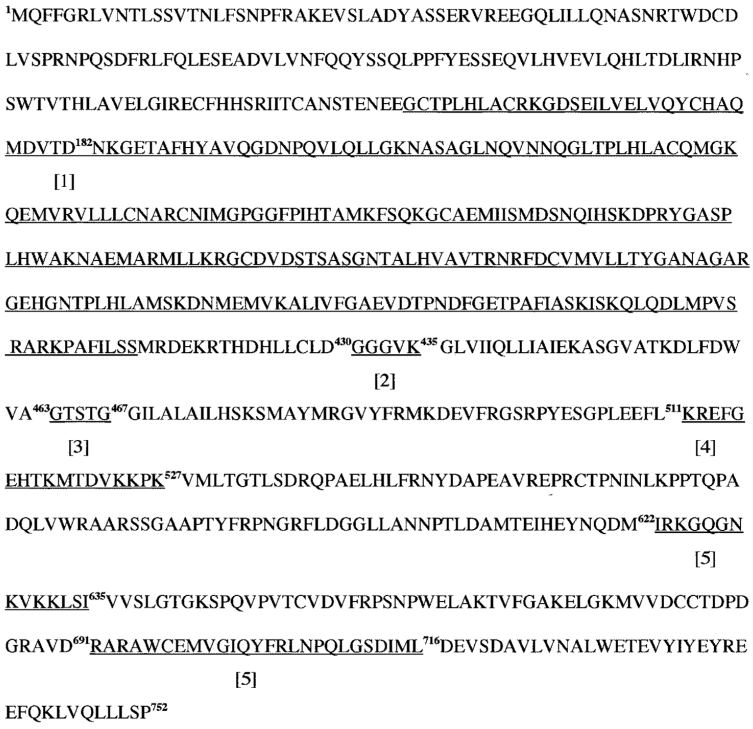

FIG. 1.

Rat pancreatic islet iPLA$_2$β-deduced amino acid sequence. The deduced 752–amino acid sequence of rat pancreatic islet iPLA$_2$β, updated from our earlier illustration (4), is shown to contain an underlined region of amino acid sequences homologous to a repetitive motif in ankyrin, in addition to a caspase-3 cleavage site, an ATP-binding domain, a catalytic serine lipase consensus sequence, a bipartite nuclear localization consensus sequence, and calmodulin-binding domain(s), which are underlined and numbered 1–5, respectively.

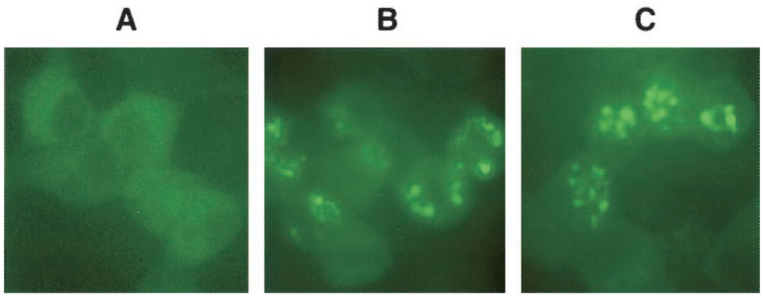

FIG. 2.

Stimulated translocation of iPLA$_2$β to the perinuclear region of INS-1 cells. INS-1 cells overexpressing iPLA$_2$β as a fusion protein with enhanced green fluorescence protein (EGFP) were stimulated with glucose in the absence and presence of forskolin, as described (41). After a stimulation period of 30 min, the cells were examined by fluorescence microscopy to monitor green fluorescence associated with EGFP. *A*: Control cells. *B*: Cells treated with glucose (2 mmol/l) alone. *C*: Cells treated with both glucose (2 mmol/l) and forskolin (2.5 μmol/l). After stimulation, punctate perinuclear accumulation of fluorescence that reflects the location of iPLA$_2$β-EGFP is evident.

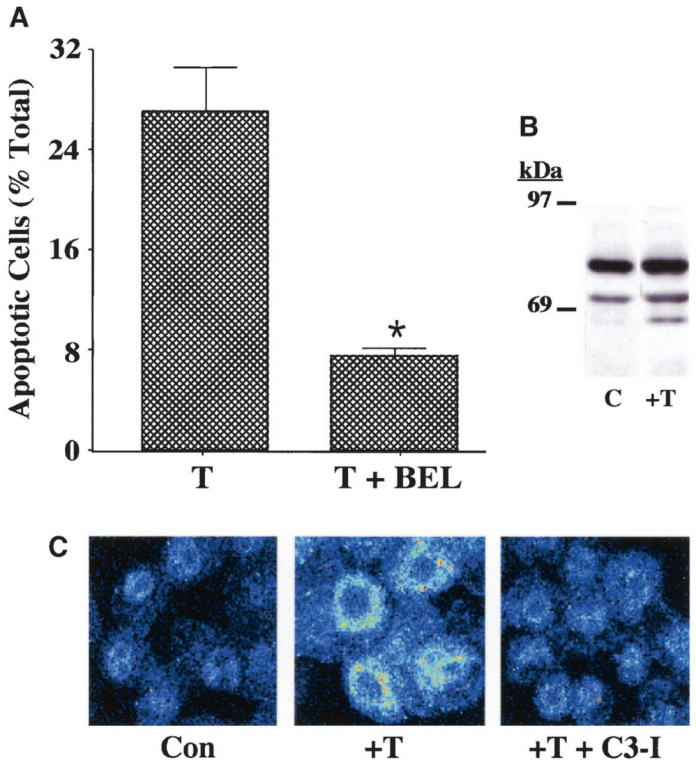

FIG. 3.

ER stress–induced apoptosis of INS-1 cells. *A*: Suppression of thapsigargin-induced apoptosis of iPLA$_2$β-overexpressing INS-1 cells by BEL. INS-1 cells stably transfected with a vector containing iPLA$_2$β cDNA (OE) construct were treated with thapsigargin (T) (1 μmol/l) for 24 h in the absence or presence of BEL (10 μmol/l). The cells were then harvested for TUNEL (Tdt-mediated dUTP nick end labeling) analyses to assess the magnitude of cell apoptosis (*T + BEL–treated group significantly different from T alone–treated group, $P < 0.05$). *B*: Immunoblotting analyses. Aliquots of INS-1 cell protein (50 μg), prepared from OE cells treated with dimethylsulfoxide (*lane 1*) or thapsigargin (1 μmol/l) (*lane 2*), were analyzed by SDS-PAGE (7.5%) and transferred onto an Immobolin-P polyvinylidene difluoride membrane. The electroblot was probed with piPLA$_2$β antibodies, and immunoreactive protein bands were visualized by enhanced chemiluminescence, as described (41). C, control. *C*: Effects of caspase-3 inhibition on iPLA$_2$β subcellular distribution. iPLA$_2$β OE INS-1 cells seeded in glass chambered slides were pretreated with caspase-3 inhibitor (C3-I) (500 nmol/l) or vehicle for 24 h. The cells were then treated with thapsigargin in the absence and presence of C3-I for 24 h and subsequently processed for iPLA$_2$β immunofluorescence analyses by confocal microscopy. Con, control.

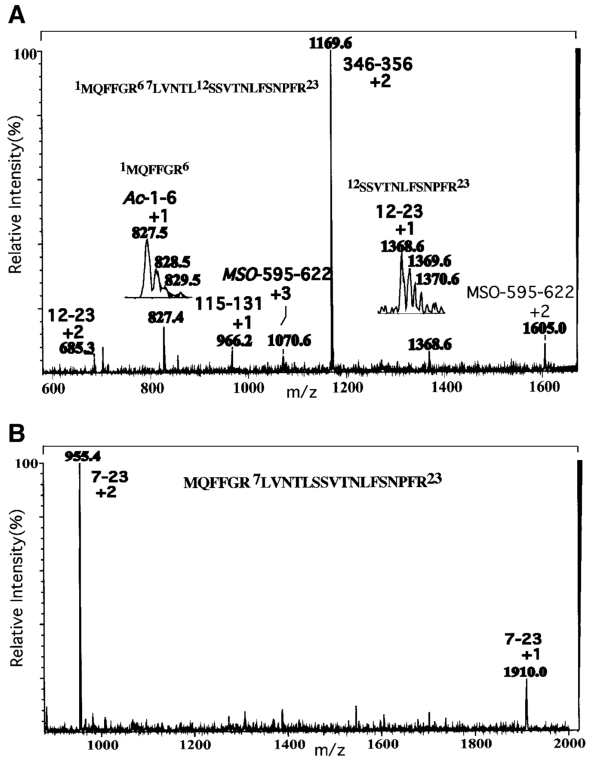

FIG. 4.

Liquid chromatography/electrospray ionization/mass spectrometry (LC/ESI/MS) of tryptic peptide of recombinant iPLA$_2$β. Recombinant iPLA$_2$β purified by sequential chromatography was digested with trypsin, and an aliquot was analyzed by LC/ESI/MS. *A*: Evidence for acetylated NH_2-terminus and NH_2-terminally truncated variant of iPLA$_2$β. *B*: Evidence for sequence proximal to residue 12.