Characterization of an ATP-Stimulatable Ca^{2+}-Independent Phospholipase A_2 from Clonal Insulin-Secreting HIT Cells and Rat Pancreatic Islets: A Possible Molecular Component of the β-Cell Fuel Sensor†

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ABSTRACT: Isolated pancreatic islets from rats and humans express a plasmalogen-preferring ATP-stimulatable, Ca^{2+}-independent phospholipase A_2 (ASCI-PLA_2) enzyme which participates in the glucose-stimulated hydrolysis of arachidonate from membrane phospholipids and in insulin secretion. Here we report that clonal insulin-secreting HIT β-cells contain substantial amounts of endogenous plasmalogens and express a similar ASCI-PLA_2 activity with the following properties: (1) Enzymatic activity as well as glucose-induced eicosanoid release and insulin secretion are inhibited by a mechanism-based suicide substrate directed towards ASCI-PLA_2. (2) HIT cell ASCI-PLA_2 is selectively activated and protected against thermal denaturation by ATP. (3) The magnitude of ASCI-PLA_2 activation by the nonhydrolyzable ATP analog AMP-PCP is similar to that by ATP. (4) The ATP concentrations required to activate ASCI-PLA_2 fall within physiologic ranges in the presence of Mg^{2+}. (5) ADP induces a concentration-dependent attenuation of the activation of ASCI-PLA_2 by ATP. HIT cell ASCI-PLA_2 exhibited an apparent isoelectric point of 7.5 on chromatofocusing analysis and was quantitatively adsorbed to an ATP–agarose matrix and selectively desorbed from this column by ATP. Mono-Q anion-exchange analysis of the active ATP–agarose eluant yielded a peak of ASCI-PLA_2 activity associated with a single protein band with an apparent molecular mass of 40 kDa. Similar chromatographic behavior of the rat pancreatic islet ASCI-PLA_2 activity was observed during sequential ATP–agarose and Mono-Q anion-exchange steps. These results indicate that HIT cells express an ASCI-PLA_2 similar to the analogous islet enzyme and suggest that expression of this enzyme and of its preferred plasmalogen substrates may be a general property of insulin-secreting β-cells.

D-Glucose induces both hydrolysis of arachidonate from pancreatic islet membrane phospholipids and the secretion of insulin (Turk et al., 1986; Wolf et al., 1986, 1991). Phospholipid hydrolysis is thought to be required for the secretory process, in part because inhibition of phospholipid hydrolysis suppresses glucose-induced insulin secretion (Laychock, 1982; Dunlop & Larkins, 1984; Metz, 1987, 1988, 1991; Metz & Dunlop, 1990; Konrad et al., 1992a,b, 1993; Ramanadham et al., 1993c). The nature of the signal from D-glucose which induces phospholipid hydrolysis in islets is not known, but Ca^{2+} influx is not an obligate event (Wolf et al., 1991; Turk et al., 1992). Metabolism of glucose within β-cells is necessary for both the induction of phospholipid hydrolysis and of insulin secretion (Malaisse et al., 1979; Ashcroft, 1980; Hedeskov, 1980; Metz, 1985; Meglasson & Matschinsky, 1986). It has been proposed that ATP generated in glycolysis may serve as a second messenger of glucose-induced events in the β-cell (Ashcroft et al., 1984; Cook & Hales, 1984; Rorsman & Trube, 1985; Sturgess et al., 1985; Cook et al., 1988).

Islets isolated from rat and human pancreata express an ATP-stimulatable Ca^{2+}-independent phospholipase A_2 (ASCI-PLA_2), which preferentially hydrolyzes arachidonate from the *sn*-2 position of phospholipids and prefers plasmenyl over diacyl substrates (Gross et al., 1993). This preference for plasmenyl substrates facilitated the utilization of a haloenol lactone suicide substrate (HELSS), which sterically resembles plasmalogens. This compound selectively inactivates islet ASCI-PLA_2 (Gross et al., 1993) at concentrations which do not influence Ca^{2+}-dependent phospholipases from several sources (Hazen et al., 1991). Inhibition of islet ASCI-PLA_2 activity with HELSS also suppresses a glucose-induced rise in cytosolic [Ca^{2+}] of β-cells and eicosanoid release and insulin secretion from intact islets (Ramanadham et al., 1993c). In addition, the arachidonate content of endogenous plasmenylethanolamine species declines when islets are stimulated with glucose (Ramanadham et al., 1993b), suggesting that glucose induces hydrolysis of arachidonate from these phospholipids.

These results suggest that glucose-induced insulin secretion from pancreatic islets involves activation of an ASCI-PLA_2 which liberates nonesterified arachidonate from β-cell plasmalogen membrane phospholipids and that this amplifies glucose-induced insulin secretion. Since ASCI-PLA_2 may represent a component of the β-cell fuel sensor apparatus, molecular characterization of this enzyme is an important objective but one that is difficult to achieve with the small number of islets that can be feasibly isolated.

Clonal insulin-secreting β-cell lines which proliferate in culture represent a more suitable source of the relatively large amounts of enzyme required for chromatographic charac-

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terization. Demonstration of expression of ASCI-PLA_2 by such cell lines would also constitute an important verification of the hypothesis that the enzyme resides specifically in β-cells rather than in other cell populations within pancreatic islets. HIT clonal β-cells, derived from the Syrian hamster (Santerre et al., 1981), express a glucose-responsive insulin secretory pathway (Hill & Boyd, 1985; Ashcroft et al., 1986; Lambert et al., 1986; Meglasson et al., 1987), similar to pancreatic islets (Ramanadham et al., 1993c). The present study was therefore undertaken to determine whether HIT cells could be exploited as a source of ASCI-PLA_2.

EXPERIMENTAL PROCEDURES

Materials. Clonal insulin-secreting HIT cells were obtained from ATCC, NIH (Bethesda, MD), and cultured at the Tissue Culture Support Center (TCSC), Washington University School of Medicine (St. Louis, MO). Tissue culture media and constituents were purchased from Gibco (Grand Island, NY). Chromatography columns and resins and PhastGel gradient 10–15 components were from Pharmacia (Piscataway, NJ). Spectra/Por 1 dialysis tubing was from Baxter (Earth City, MO), and components for PAGE analyses were from BioRad (Richmond, CA). Phospholipid standards were from Avanti Polar Lipids (Birmingham, AL) and the HPLC columns from Alltech (Deerfield, IL). [$^3H_8$]Arachidonate (100 Ci/mmol) and [^{125}I]Bolton–Hunter reagent (4400 Ci/mmol) were from Dupont New England Nuclear (Boston, MA). Protein MW standards and diacyl phospholipids [(16:0a/18:1)-PC, (16:0a/20:4)-PC, and (16:0a/20:4)-PE] containing [^{14}C]-labeled *sn*-2 substituents (specific activity = 1.1×10^2 dpm/pmol) were from Amersham (Arlington Heights, IL). The plasmenylcholine substrate containing [^{3}H]-labeled oleate as the *sn*-2 substituent (16:0p/18:1-PC; specific activity = 1.1×10^3 dpm/pmol) and HELSS[1] were prepared as previously described (Hazen et al., 1991).

Media. The required media were constituted as follows: KRB (Krebs–Ringer bicarbonate buffer; 25 mM Hepes, pH 7.4, 115 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 3 mM D-glucose, pH 7.3); assay buffer (200 mM Tris-HCl, pH 7.5, 37 °C); and homogenization buffer for studies with crude enzyme (A, 250 mM sucrose and 40 mM Tris–HCl, pH 7.1, 4 °C). Buffers for enzyme purification included the following (B) homogenization buffer (250 mM sucrose, 10 mM imidazole, 10 mM KCl, pH 7.8, 4 °C); (1) pre-DEAE dialysis buffer (15 mM imidazole, 5 mM K_2HPO_4, 10% glycerol, pH 7.8, 4 °C); (2) DEAE column elution buffer (10 mM imidazole, 10 mM KCl, 100 mM NaCl, 10% glycerol, 1 mM DTT, pH 8.0, 4 °C); (3) pre-CF dialysis buffer (10 mM imidazole, 10 mM KCl, 25% glycerol, 1 mM DTT, pH 8.4, 4 °C); (4) CF column elution buffer (10% poly buffer 96, 5% poly buffer 74, 25% glycerol, 1 mM DTT, pH 8.4, 4 °C); (5) ATP column elution buffer (10 mM imidazole, 25% glycerol, 1 mM DTT, pH 8.3, 4 °C); (6A) Mono Q buffers (50 mM imidazole, 25% glycerol, 1 mM DTT, pH 8.3, 4 °C); and (6B) 6A + 500 mM NaCl.

[1] Abbreviations: ASCI, ATP-stimulatable Ca^{2+}-independent; BSA, bovine serum albumin; DMA, dimethyl acetal; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; PLA_2, phospholipase A_2; FAME, fatty acid methyl ester; GC, gas chromatography; HBSS, Hank's balanced salt solution; HELSS, (*E*)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high-performance liquid chromatography; KRB, Krebs–Ringer bicarbonate buffer; MS, mass spectrometry; NP, normal phase; PC, choline–phospholipids; PCI, positive ion chemical ionization; PE, ethanolamine–phospholipids; PGE_2, prostaglandin E_2; RP, reversed phase.

Table 1: Expression of a Ca^{2+}-Independent Phospholipase A_2 Activity[a] by Clonal Insulin-Secreting HIT Cells

	specific activity of phospholipase A_2 (pmol·mg protein^{-1} min^{-1})		
	10 mM $CaCl_2$	10 μM $CaCl_2$	10 mM EGTA
cytosol	68 ± 12 (14)	116 ± 21 (5)	185 ● 18 (65)
microsomes	196 ± 33 (14)	385 ± 63 (5)	317 ± 49 (61)

[a] Phospholipase A_2 activity was measured in media containing either 10 mM Ca^{2+}, 10 μM Ca^{2+}, or zero-Ca^{2+} and 10 mM EGTA. Reactions were initiated by injection of the plasmenylcholine substrate containing [^{3}H]oleate [(16:0p/18:1-PC)] prepared in ethanol (2.5 μM in 5 μL). Assay mixtures were incubated at 37 °C for 3 min, and the assay was terminated with the addition of *n*-butanol (100 μL). Assay mixtures were centrifuged (200*g* for 4 min), and the reaction products in 25 μL of the upper butanol layer were separated by thin-layer chromatography (running buffer: acetic acid/ethyl ether/petroleum ether, 1/20/80, v:v). The hydrolyzed [^{3}H]oleate region was identified with iodine vapor and quantified by liquid scintillation spectrometry. Approximately 15–26% of the added substrate was hydrolyzed by the HIT cell Ca^{2+}-independent PLA_2 activity under these assay conditions. Protein concentrations were determined by Bio-Rad assay, and the radiolabeled product release was converted to a specific PLA_2 activity measurement, as previously described (Gross et al., 1993). The data represent mean ± SEM for the (*n*) values.

Table 2: Substrate Preference of HIT Cell Ca^{2+}-Independent Phospholipase A_2[a]

	specific activity of Ca^{2+}-independent PLA_2 (pmol·mg protein^{-1} min^{-1})	
substrate	concn (μM)	cytosol
(16:0p/18:1)-PC	2.5	185 ● 18
	10.0	266 ● 50
(16:0a/18:1)-PC	2.5	8 ± 1
	10.0	21 ± 11
(16:0a/20:4)-PC	2.5	50 ± 6
	10.0	65 ± 6
(16:0a/20:4)-PE	2.5	146 ± 14
	10.0	114 ± 15

[a] Calcium-independent PLA_2 activity was assayed in HIT cell cytosol as described in Table 1. Reactions were carried out in buffer containing 10 mM EGTA and no added Ca^{2+} and initiated with injection of the substrates prepared in ethanol (5 μL): 1-*O*-(*Z*)-hexadec-1′-enyl-2-[9,10-[$^3H_2$]]octadec-9′-enoyl-*sn*-glycero-3-phosphocholine [(16:0p/18:1)-PC]; 1-hexadecanoyl-2-[1-[^{14}C]]octadec-9′-enoyl-*sn*-glycero-3-phosphocholine [(16:0a/18:1)-PC]; 1-hexadecanoyl-2-[1-[^{14}C]]eicosa-5′,8′,11′,14′-tetraenoyl-*sn*-glycero-3-phosphocholine [(16:0a/20:4)-PC]; or 1-hexadecanoyl-2-[1-[^{14}C]]eicosa-5,8,11,14-tetraenoyl-*sn*-glycero-3-phosphoethanolamine [(16:0a/20:4)-PE]. The assay and specific enzymatic activity measurement procedures were as described in Table 1. The data are the mean ± SEM (n = 3–65).

Preparation of Subcellular Fractions of HIT Cells. HIT cells (7×10^8 for enzymatic activity studies or 4×10^{10} for chromatographic analyses) were detached from T-175 flasks with 0.05% trypsin/0.02% EDTA, washed with minimum essential medium, gently pelleted, and sonicated in homogenization buffer B. Cytosolic and microsomal fractions were then prepared as described (Gross et al., 1993).

Phospholipase A_2 Enzymatic Activity Measurements. Cytosolic (150 μL, ca. 75 μg) and microsomal (100 μL, ca. 60 μg) fractions were incubated with radiolabeled phospholipid substrate under various experimental conditions (total assay volume 400 μL), as specified in the figures and tables. Details of assay protocol are indicated in the Table 1 legend. An assay of Ca^{2+}-independent PLA_2 activity following chromatographic analyses was performed using 50–100 μL of fraction aliquots (total assay volume 200 μL).

Specific Enzyme Assays. PLA_2 activity was measured in the presence of $CaCl_2$ (10 mM or 10 μM) or 10 mM EGTA and no added Ca^{2+} (zero-Ca^{2+}) using the [^{3}H]-labeled plasmenylcholine substrate [(16:0p/18:1)-PC], where "p"

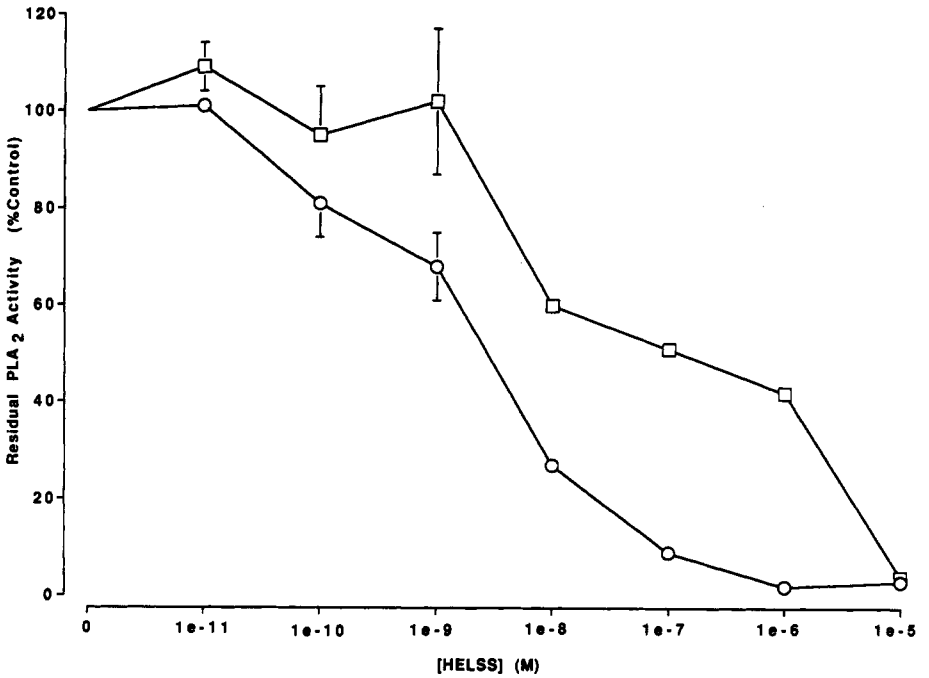

FIGURE 1: Concentration-dependent inhibition of Ca^{2+}-independent phospholipase A_2 activity in HIT cells by HELSS. Cytosolic or microsomal enzyme was first incubated at room temperature for 2 min in the presence of media containing no added Ca^{2+}, 10 mM EGTA, and varying concentrations of HELSS (10^{-11}–10^{-5} M). Enzymatic activity was assayed with the plasmenylcholine substrate containing [^{3}H]oleate (16:0p/18:1)-PC as described in Table 1 and is plotted as a function of [HELSS] in the assay mixture. The plotted values represent means of data obtained from four experiments each in cytosol (squares) and microsomes (circles). The calculated IC_{50} values in the cytosol and microsomes were 2.26 nM and 1.29 nM, respectively.

denotes an *sn*-1 plasmenyl linkage. Substrate preference of the cytosolic Ca^{2+}-independent PLA_2 activity was examined by comparing the rate of hydrolysis of (16:0p/18:1)-PC, (16:0a/18:1)-PC, (16:0a/20:4)-PC, and (16:0a/20:4)-PE, where "a" denotes an *sn*-1 acyl linkage.

The Ca^{2+}-independent PLA_2 activity was further characterized under zero-Ca^{2+} conditions using the plasmenylcholine substrate [(16:0p/18:1)-PC] in the presence of either HELSS (10^{-11}–10^{-5} M), various nucleotides and phosphates, 1 mM ATP ± 10 mM $MgCl_2$, or ADP (1 μM–1 mM) ± 1 mM ATP. Thermal lability of the Ca^{2+}-independent PLA_2 activity was examined in assays in which the cytosolic enzyme was first preincubated for 20 min at 37 °C either in the absence of ATP or in the presence of 10 mM ATP.

Analyses of HIT Cell Phospholipid Content. HIT cells (7.0×10^8) were incubated overnight with [^{3}H$_8$]arachidonate (100 μCi) prior to extraction of the phospholipids with chloroform/methanol (1:1, v:v) under neutral conditions (Bligh & Dyer, 1959) and separated into head-group classes by normal-phase HPLC as described (Ramanadham et al., 1993b). The PE and PC peaks were collected separately and then analyzed by reversed-phase (RP)-HPLC (Patton et al., 1982; Chilton & Murphy, 1986; DaTorre & Creer, 1991). Isolated phospholipids were then subjected either to acid methanolysis or to saponification and then analyzed by chemical ionization GC–MS in either the positive ion or the negative ion mode, as described (Ramanadham et al., 1993a,b).

Measurement of Glucose-Stimulated Insulin Secretion and PGE_2 Release from HIT Cells. HIT cells (500 000/well) subcultured in a 24-well plate for 3 days were washed with KRB medium containing no glucose (zero-KRB) and incubated for 30 min in the same medium. Next, the cells were incubated for 30 min with KRB medium containing 17 mM glucose and HELSS (20 μM) or vehicle (0.3% EtOH). The cells were then washed three times with zero-KRB medium containing 0.1% free fatty acid-free BSA to remove the HELSS and then incubated in zero-KRB containing 0.1% BSA for 30 min. The medium was discarded, and the cells were incubated for 45 min in the presence of either 0 or 17 mM glucose. All incubations were performed at 37 °C under an atmosphere of 5% CO_2/95% air with gentle shaking. Insulin and PGE_2 contents in the incubation media were measured as described (Wright et al., 1971; Ramanadham et al., 1993c). Whereas nonesterified arachidonate largely remains within the β-cell, PGE_2, an arachidonate metabolite, is released into the medium and therefore serves as a readily measurable marker of arachidonate hydrolysis from membrane phospholipids (Wolf et al., 1991).

HIT Cell Cytosolic Ca^{2+}-Independent PLA_2 Purification. Crude cytosolic supernatant fraction was dialyzed against buffer 1 and subjected to sequential chromatographic analyses (DEAE Sephacel anion-exchange, chromatofocusing, ATP–agarose, and Mono Q anion-exchange columns) at 4 °C over a 3-day period.

Dialyzed HIT cell cytosolic protein was loaded onto a DEAE column (1.5 × 8 cm) and washed with buffer 1 containing 1 mM DTT until the A_{280} returned to base line. Buffer 2 containing 100 mM NaCl was then applied to the column to elute the Ca^{2+}-independent PLA_2. Fractions containing the enzymatic activity were dialysed and then applied to a PBE-94 chromatofocusing column (1 × 8 cm, 1.8 mL/min). The column was washed with buffer 3, and the Ca^{2+}-independent PLA_2 was eluted using a shallow pH gradient generated with 10% polybuffer 96, 5% polybuffer 74, 25% glycerol, 1 mM DTT, pH 6.9. Recovered enzymatic activity was immediately applied to a 1- × 1-cm *N*6-[(6-aminohexyl)carbamomethyl]-ATP–agarose column. The column was sequentially washed with buffer 5, buffer 5 containing 10 mM AMP, buffer 5, and buffer 5 containing 1 mM ATP. The Ca^{2+}-independent PLA_2 activity desorbed with ATP was applied onto a Mono Q column and was eluted from this column utilizing a linear salt gradient

(buffers 6A + 6B) on a SMART liquid chromatography system (Pharmacia, Piscataway, NJ).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Autoradiography of HIT Cell Cytosolic Ca^{2+}-Independent Phospholipase A_2. An aliquot (100 μL) of fractions recovered from the Mono Q column containing Ca^{2+}-independent PLA_2 activity was incubated with 250 μCi of [^{125}I]Bolton–Hunter reagent overnight at 4 °C. An aliquot of the radioiodinated sample was diluted in sample buffer (1 M Tris–HCl, pH 6.8, 69 mM SDS, 10% glycerol, 0.01% bromophenyl blue, 1% 2-mercaptoethanol), boiled for 5 min, and applied to a 10% SDS–PAGE gel (Laemmli, 1970). After overnight fixation (H_2O/MeOH/acetic acid, 5:5:1, v:v:v), the gel was dried and the protein bands were visualized by autoradiography.

Purification of Rat Pancreatic Islet Ca^{2+}-Independent Phospholipase A_2. Islet cytosol was prepared from islets isolated aseptically from 60 male Sprague–Dawley rats as described (Gross et al., 1993). The cytosol was dialyzed overnight against buffer 5 and applied to a ATP–agarose column. The Ca^{2+}-independent PLA_2 activity desorbed from the column with ATP was applied to a Mono Q column and eluted utilizing a linear salt gradient. The low starting amounts of protein and activity precluded analyses by anion-exchange or chromatofocusing steps.

RESULTS

Calcium-Independent PLA_2 Activity in HIT Cells: Demonstration of its Expression, Substrate Preference, and Susceptibility to Inhibition by HELSS. Significant Ca^{2+}-independent PLA_2 activity was expressed in both HIT cell cytosolic and membrane fractions in assay medium containing zero Ca^{2+} and the plasmalogen substrate (16:0p;18:1)-PC (Table 1). Cytosolic PLA_2 activity measured in zero-Ca^{2+} was greater than the activity measured in the presence of 10 μM Ca^{2+}, while in the microsomes similar activity was measured under these two conditions.

Examination of the substrate preference of the HIT cell Ca^{2+}-independent PLA_2 revealed (Table 2) that the HIT cell enzyme exhibited (a) a clear preference for plasmalogen [(16:0p/18:1)-PC] versus the corresponding diacyl [(16:0a/18:1)-PC] substrate, (b) a preference for arachidonate [(16:0a/20:4)-PC] versus oleate [(16:0a/18:1)-PC] as the *sn*-2 substituent, and (c) a preference for ethanolamine [(16:0a/20:4)-PE] over choline [(16:0a/20:4)-PC] head groups.

In view of the preference of HIT cell Ca^{2+}-independent PLA_2 enzyme for plasmalogen substrates, the susceptibility of its activity to inhibition by HELSS was examined. As illustrated in Figure 1, pretreatment of HIT cell cytosol and microsomes with HELSS resulted in a concentration-dependent inhibition of activity in both fractions.

Demonstration of Endogenous Plasmalogen Phospholipid Molecular Species in HIT Cells. Since the HIT cell Ca^{2+}-independent PLA_2 activity exhibited a preference for arachidonate-containing plasmenylethanolamine substrates, the endogenous HIT cell content of phospholipids with these structural features was examined. Phospholipids extracted from [$^3H_8$]arachidonate-loaded HIT cells were first separated into phospholipid head group classes by NP-HPLC, and the ethanolamine phospholipids were subsequently analyzed by RP-HPLC, as described (Ramanadham et al., 1993a,b). Such analyses revealed six major arachidonate-containing species (Figure 2, upper panel).

A feature of plasmalogens that can be exploited in their identification is the lability of the alkenyl moiety upon exposure

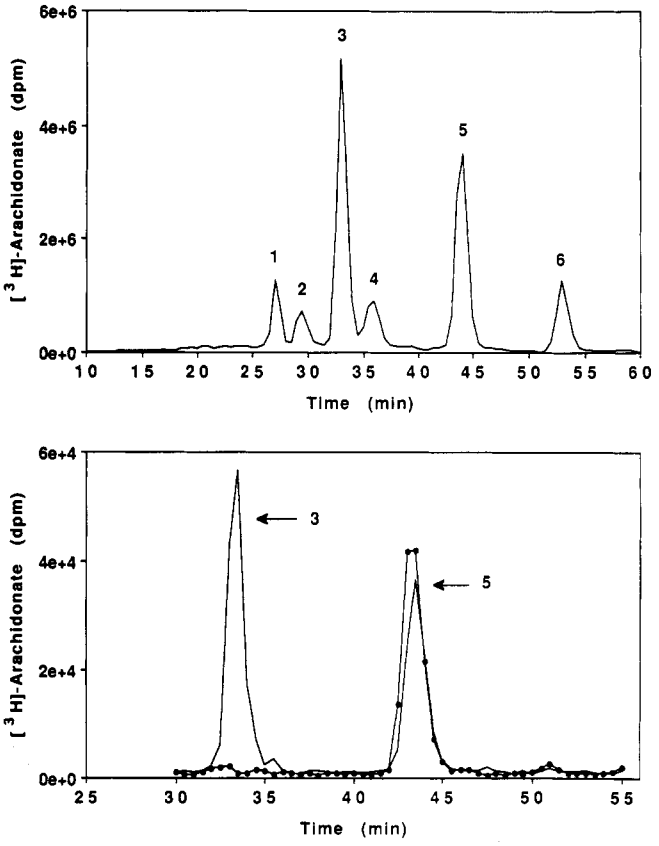

FIGURE 2: Reversed-phase (RP)-HPLC analyses of ethanolamine phospholipids from HIT cells. Phospholipids were extracted from HIT cells and separated into head group classes by normal-phase HPLC as described in the Experimental Procedures. The PE peak was then harvested and analyzed by RP-HPLC under conditions where molecular species of phospholipids are separated. Upper panel: RP-HPLC analysis of PE. Lower panel: acid-lability of plasmenylethanolamine molecular species. PE peaks 3 and 5 were collected separately, combined, and split into two aliquots. One aliquot was treated with methanolic 1 N HCl/chloroform (1/1) (acid-treatment) and the other with methanol/chloroform (sham-treatment), as described in the Experimental Procedures. Each aliquot was then reanalyzed by RP-HPLC. The tracings represent the sham-treated chromatogram (no symbol) and the acid-treated chromatogram (closed symbols).

to acid under conditions in which the diacyl- and alkylacyl-phospholipids are stable (DaTorre & Creer, 1991). As illustrated in Figure 2 (lower panel), following acid treatment PE peak 3 was found to be acid-labile and peak 5 was acid-stable. Similar analyses of the remaining PE peaks revealed that peaks 4 and 6 were also acid-labile while peaks 1 and 2 were acid-stable (data not presented). In addition, analyses of the PC peaks did not reveal any significant acid-labile peaks (data not presented).

Another distinguishing feature of plasmalogens is that under conditions of acid methanolysis, the *sn*-1 fatty aldehyde residues are released as dimethyl acetals (DMA), whereas the acylated fatty acid residues are released as fatty acid methyl esters (FAME) (Gross, 1984). GC–PCI–MS analyses of the acid methanolysis products from the individual PE peaks (data not shown) revealed that the acid-labile peaks 3, 4, and 6 contained the plasmenyl species (C16:0p/C20:4)-PE, (C18:1p/C20:4)-PE, and (C18:0p/C20:4)-PE, respectively, and that the acid-stable peaks 1, 2, and 5 contained the diacyl species (C16:0a/C20:4)-PE, (C18:1a/C20:4)-PE, and (C18:0a/C20:4)-PE, respectively. Upon saponification and stable isotope dilution GC-MS analyses (Ramanadham et al., 1993a,b), the three plasmenylethanolamine molecular species were found to contain 44% of the total arachidonate mass present among

Table 3: Glucose-Stimulated Insulin Secretion and Eicosanoid Release from HIT Cells[a]

[glucose] (mM)	insulin secretion (ng/mg protein)		PGE_2 (pg/mg protein)	
	control[b]	+HELSS[c]	control	+HELSS
0	84 ± 18	92 ± 22	767 ± 96	356 ± 20
17	270 ± 19	99 ± 26[d]	2753 ± 105	669 ± 30[d]

[a] The insulin and PGE_2 contents in incubation media were determined by RIA and EIA, respectively. [b] Vehicle-pretreated control group. [c] HELSS-pretreated group. The data represent mean ± SEM (n = 3). [d] HELSS-pretreated group significantly different from the control group, $p < 0.05$.

the six identified PE molecular species. This distribution of arachidonate between the plasmalogen and diacylethanolamine phospholipids is identical to that reported earlier in the rat pancreatic islet (Ramanadham et al., 1993b).

Glucose-Stimulated Insulin Secretion and Eicosanoid Release from Intact HIT Cells. The role of the HIT cell Ca^{2+}-independent PLA_2 in glucose-induced insulin secretion and eicosanoid release from HIT cells was examined next. Both insulin secretion and PGE_2 release from HIT cells were stimulated nearly 3.5-fold over basal levels by 17 mM glucose (Table 3). In HIT cells pretreated with HELSS, glucose-stimulated insulin secretion was nearly abolished (stimulated values–basal levels; control 186 ± 19 vs + HELSS, 7 ± 26 ng/mg protein), and PGE_2 release was reduced by 85% (control, 1986 ± 105 vs + HELSS, 327 ± 27 pg/mg protein).

Activation of HIT Cell Ca^{2+}-Independent Phospholipase A_2 by ATP. The ability of ATP to activate the HIT cell Ca^{2+}-independent PLA_2 was examined in assays in which the cytosol and microsomes were incubated in the presence of varying concentrations of exogenous ATP. As illustrated in Figure 3 (left panel), Ca^{2+}-independent PLA_2 activity measured in both cytosol and microsomes exhibited an ATP concentration-dependent rise, indicating that the HIT cells express an ATP-stimulatable Ca^{2+}-independent phospholipase A_2 (ASCI-PLA_2) activity.

Table 4: Influence of Various Nucleotides on HIT Cell Cytosolic Ca^{2+}-Independent Phospholipase A_2[a] Activity

	ratio of enzymatic specific activity (+nucleotide/–nucleotide)
Triphosphates	
ATP	2.38
AMP-PCP	2.27
GTP	0.87
CTP	1.23
Diphosphates	
ADP	1.24
GDP	1.09
Monophosphates	
AMP	1.08
GMP	0.25

[a] Calcium-independent PLA_2 activity was measured in HIT cell cytosol with the plasmenylcholine substrate containing [^{3}H]oleate (16:0p/18:1)-PC. The assay and specific enzymatic activity calculations were as described in Table 1. The data represent stimulation of Ca^{2+}-independent PLA_2 activity in the presence of nucleotides (10 mM) relative to Ca^{2+}-independent PLA_2 activity measured in the absence of nucleotides (n = 2–11).

As illustrated in Figure 3 (left panel), stimulation of HIT cell ASCI-PLA_2 activity by ATP was evident in the micromolar range. Under physiological conditions, a large fraction of ATP in intact cells exists as a $MgATP^{2-}$ complex (O'Rourke et al., 1992). Addition of Mg^{2+} resulted in a rightward shift in the ATP concentration-dependent enzymatic activation curve (i.e., to higher ATP concentrations) (Figure 3, right panel), and the $EC_{50\%}$ was increased by 1 order of magnitude (from ~0.1 to ~1 mM).

Characterization of Nucleotide-Regulation of HIT Cell ASCI-PLA_2. As summarized in Table 4, the Ca^{2+}-independent PLA_2 activity was stimulated similarly by ATP and AMP-PCP (a nonhydrolyzable analog of ATP) but not by the other nucleotides tested. A second feature of the interaction of ATP with the HIT cell ASCI-PLA_2 enzyme is that it confers stability to the enzymatic activity against thermal inactivation

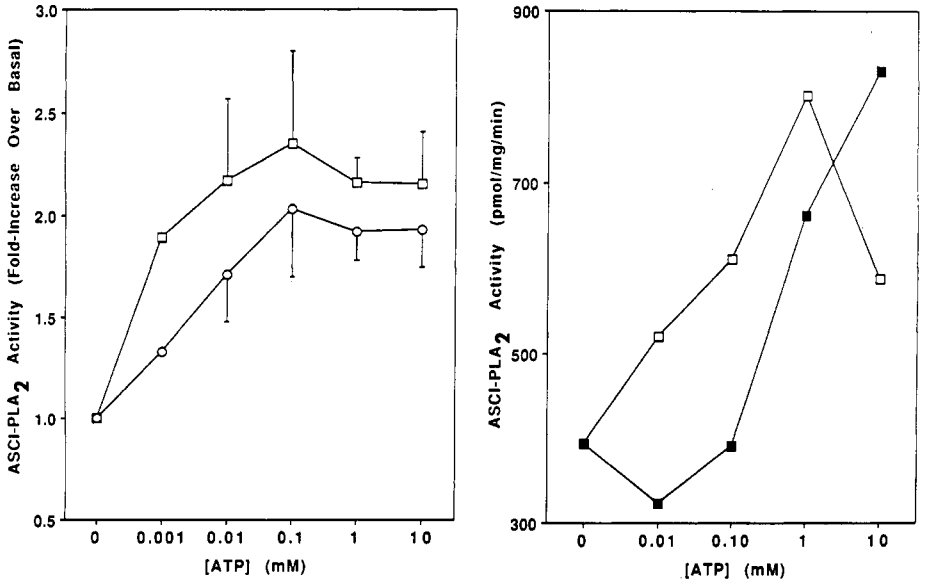

FIGURE 3: Activation of Ca^{2+}-independent phospholipase A_2 activity in HIT cells by ATP and Mg^{2+}-ATP. Ca^{2+}-independent enzymatic activity measured with the plasmenylcholine substrate containing [^{3}H]oleate [(16:0p/18:1)-PC] was performed as described in Table 1. Left panel: the reaction mixture contained varying concentrations of ATP (1 μM, 10 μM, 100 μM, 1 mM, or 10 mM). Plotted values represent mean ± SEM (cytosol, n = 50, and microsomes, n = 38) of the ratio of ASCI-PLA_2 activity in the presence of ATP relative to ASCI-PLA_2 activity in the absence of ATP in cytosol (open symbols) and microsomes (closed symbols). Basal specific activities (in the presence of EGTA alone) were 203 ± 22 and 274 ● 45 pmol/mg protein/min in cytosol and microsomes, respectively. Right panel: influence of $MgCl_2$ on ATP-activation of the HIT cell cytosolic Ca^{2+}-independent PLA_2 activity. The reaction mixture contained either 1 mM ATP without $MgCl_2$ (open squares) or with $MgCl_2$ (10 mM, closed squares). The values represent averages of duplicate measurements.

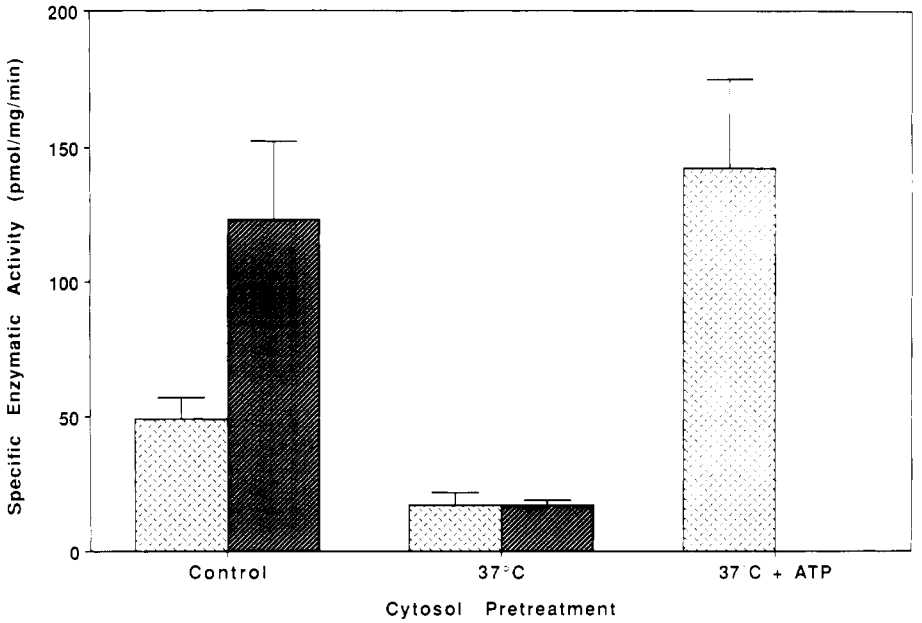

FIGURE 4: Thermal inactivation of HIT cell cytosolic ASCI-PLA_2 activity and its stabilization by ATP. Cytosol prepared from HIT cells was either kept on ice (control) or preincubated for 20 min at 37 °C either in the absence or presence of 10 mM ATP. Subsequent assay for Ca^{2+}-independent enzymatic activity with the plasmenylcholine substrate containing [^{3}H]oleate (16:0p/18:1)-PC was performed as described in Table 1. The values represent mean ± SEM (n = 6–9) of specific enzymatic activity measurements. (Stippled bars, enzymatic activity assayed in the absence of ATP, and diagonal bars, activity assayed only in the control and 37 °C groups in the presence of 10 mM ATP).

(Figure 4). Following mild heat treatment in the absence of ATP, HIT cell ASCI-PLA_2 activity in the cytosol declined significantly, as compared with activity measured in cytosol maintained at 4 °C (Figure 4). Subsequent addition of ATP during the assay was not sufficient to restore ASCI-PLA_2 activity in the heat-treated cytosol. In contrast, cytosolic ASCI-PLA_2 activity was preserved when the cytosol was preincubated at 37 °C in the presence of ATP. These results indicate that HIT cell cytosolic ASCI-PLA_2 is extremely thermally labile and that ATP can protect it against thermal inactivation. The HIT cell microsomal ASCI-PLA_2 activity was unaffected by mild heat (37 °C) treatment (data not shown). Together with the finding that the membrane-associated enzymatic activity in HIT cells, in contrast to the analogous activity in pancreatic islets (Gross et al., 1993), is also activated by ATP, these results raise the possibility that the regulatory and catalytic components of the ASCI-PLA_2 enzyme complex remain tightly associated in the HIT cell membranes.

ADP is thought to act in concert with ATP to cooperatively modulate the activity of other components of the β-cell fuel sensor apparatus, such as the K_{ATP} channel (Kakei et al., 1986; Misler et al., 1986). To determine whether ADP influenced the effects of ATP on HIT cell ASCI-PLA_2, enzymatic activity in HIT cells was measured in the presence of ADP ± ATP (Figure 5). Whereas ADP alone did not significantly alter ASCI-PLA_2 activity, ADP exerted a concentration-dependent suppression of ASCI-PLA_2 activation by ATP. Maximal inhibition observed was 60% and 80% in the cytosol and microsomes, respectively.

Analyses of HIT Cell ASCI-PLA_2 by Sequential Chromatography, SDS-PAGE, and Autoradiography. Since the properties of the HIT cell ASCI-PLA_2 described above are consistent with a role for the enzyme in mediating glucose-induced hydrolysis of arachidonate from membrane phospholipids in this clonal β-cell line, chromatographic characterization of the HIT cell ASCI-PLA_2 was undertaken.

Crude HIT cell ASCI-PLA_2 activity was first applied to a DEAE–Sephacel column and was eluted from this column as a sharp peak using a stepwise NaCl gradient (Figure 6, left panel). The recovered ASCI-PLA_2 activity was next applied to and eluted from a chromatofocusing column and exhibited an apparent isoelectric point of about 7.5 (Figure 6, right

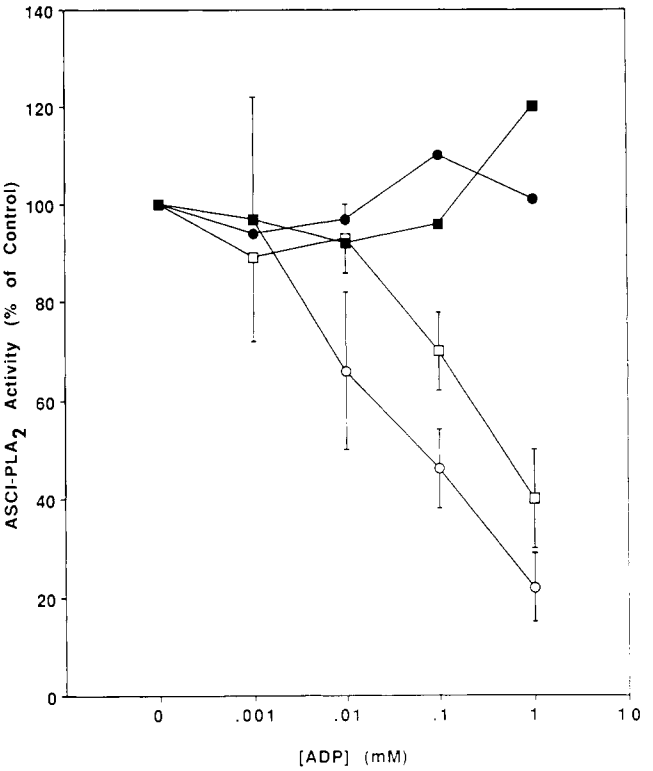

FIGURE 5: Influence of ADP on the activation of HIT cell ASCI-PLA_2 by ATP. The ASCI-PLA_2 activity was measured in the cytosolic (circles) and microsomal (squares) fractions prepared from HIT cells in media containing no added Ca^{2+}. Assay mixtures contained ADP (1 μM–1 mM) without ATP (closed symbols) or with ATP (1 mM, open symbols). Assay for ASCI-PLA_2 activity with the plasmenylcholine substrate containing [^{3}H]oleate (16:0p/18:1)-PC was performed as described in Table 1. The values represent averages of six measurements.

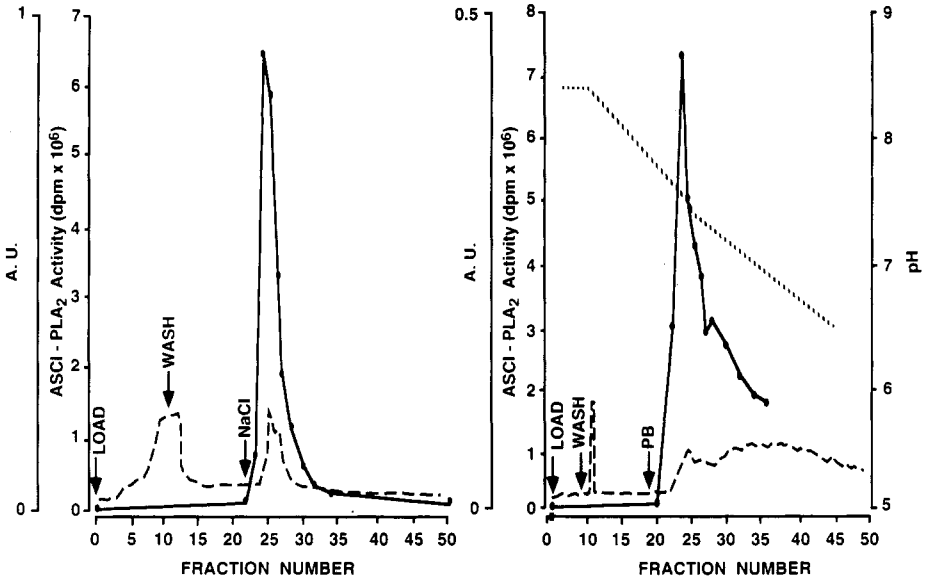

FIGURE 6: Elution of HIT cell cytosolic ASCI-PLA_2 from DEAE–Sephacel anion-exchange and chromatofocusing columns. Left panel: DEAE–Sephacel anion-exchange analysis of the ASCI-PLA_2. Dialyzed crude HIT cell cytosol was loaded onto and eluted from the DEAE–Sephacel column, and the fractions were assayed for ASCI-PLA_2 activity as described in the Experimental Procedures: [- - -, ultraviolet absorbance at 280 nm; closed symbol, ASCI-PLA_2 (dpm) activity]. Right panel: chromatofocusing analysis of the ASCI-PLA_2. Fractions from the DEAE–Sephacel column containing ASCI-PLA_2 activity were pooled, and enzymatic activity was eluted from the column described in the Experimental Procedures and as described in Table 1: [- - -, ultraviolet absorbance at 280 nm; closed symbol, ASCI-PLA_2 activity (dpm); ···, pH].

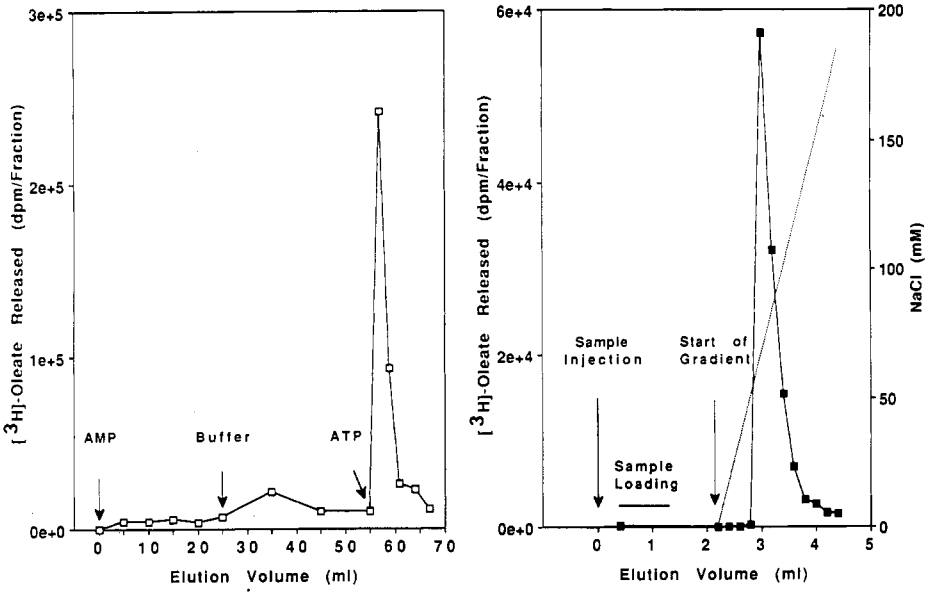

FIGURE 7: ATP-affinity chromatography and FPLC-anion exchange chromatography of HIT cell cytosolic ASCI-PLA_2. Left panel: CF fractions containing ASCI-PLA_2 activity (Figure 6B) were pooled and immediately applied to an ATP–agarose column, and the ASCI-PLA_2 activity was desorbed with ATP as described in the Experimental Procedures. Right panel: enzymatic activity recovered from the ATP column was loaded onto a HR5/5 Mono Q column. The ASCI-PLA_2 activity was eluted in 200-μL fractions from the column utilizing a linear NaCl gradient and assayed as described in Table 1: [- - -, NaCl gradient; closed symbols, ASCI-PLA_2 activity (dpm)].

panel). Protein concentrations in the eluant were too low to permit accurate measurement after this or subsequent chromatographic steps.

The interaction of ASCI-PLA_2 with ATP was exploited by applying the chromatofocusing column fractions containing the ASCI-PLA_2 activity onto an ATP–agarose column. The ASCI-PLA_2 activity was quantitatively adsorbed to this matrix and was selectively desorbed from the column with ATP (Figure 7, left panel), further illustrating the specific interaction of HIT cell ASCI-PLA_2 with ATP. As expected, the resulting interaction of the ASCI-PLA_2 and ATP during desorption precluded further activation of the partially-purified enzyme by ATP. Analogous to the crude ASCI-PLA_2, activity of the partially-purified enzyme was inhibited by HELSS (by 3%, 55%, and 93% with 10^{-11}, 10^{-9}, and 10^{-6} M HELSS, respectively).

ASCI-PLA_2 activity recovered from the ATP–agarose column was further analyzed by application onto a FPLC-Mono Q anion-exchange column utilizing a linear NaCl gradient. Peak enzymatic activity was eluted from the column with approximately 65 mM NaCl (Figure 7, right panel) and was associated with a single predominant band with an apparent molecular mass of approximately 40 kDa on SDS–PAGE analysis (Figure 8).

Chromatographic Analysis of Rat Islet ASCI-PLA_2. To determine whether ASCI-PLA_2 from HIT cells and from

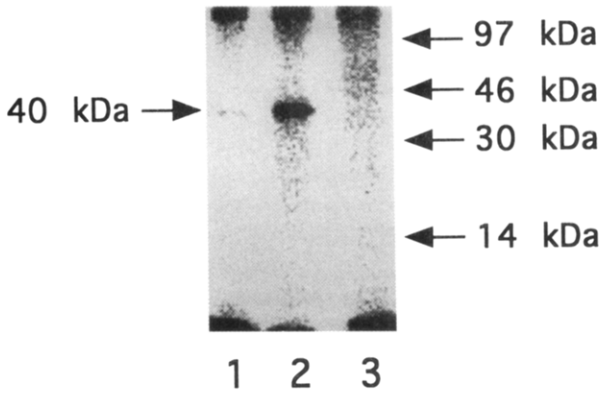

FIGURE 8: Autoradiograph of [^{125}I]-labeled HIT cell ASCI-PLA_2 resolved by SDS–PAGE. Aliquots of the active fractions from the Mono Q column (Figure 7B) were iodinated utilizing [^{125}I]Bolton–Hunter reagent, and the reaction was allowed to continue overnight at 4 °C. An aliquot of the iodinated sample was then boiled for 5 min in the presence of 100 mM 2-mercaptoethanol and 10% SDS and loaded onto a 10% polyacrylamide slab gel, electrophoresed, fixed, dried, and visualized by autoradiography as described in the Experimental Procedures. Lanes 1 and 3 represent Mono Q fractions containing minimal enzymatic activity at elution volumes of 2.8 and 3.4 mL, respectively, and lane 2 represents fraction containing peak enzymatic activity at elution volume of 3.0 mL. Molecular weight standards are indicated on the right.

isolated pancreatic islets exhibited similar chromatographic properties, the behavior of islet cytosolic ASCI-PLA_2 activity on ATP-affinity chromatography and on Mono-Q FPLC analysis was characterized. Islet cytosolic ASCI-PLA_2 was quantitatively adsorbed to ATP–agarose and selectively desorbed by ATP, similar to the HIT cell ASCI-PLA_2 (Figure 9, left panel). Islet ASCI-PLA_2 activity recovered from the ATP-affinity column was then subjected to Mono Q analysis and was found to elute at a salt concentration identical to that for the HIT cell enzyme (Figure 9, right panel).

DISCUSSION

D-Glucose-induced insulin secretion from pancreatic islets involves processes which require entry of D-glucose into islet β-cells and its subsequent metabolism (Malaisse et al., 1979; Ashcroft, 1980; Hedeskov, 1980; Meglasson & Matschinsky, 1986). ATP generated by the glycolytic pathway inactivates a β-cell plasma membrane K^+-channel (K_{ATP}) (Ashcroft et al., 1984; Cook & Hales, 1984; Rorsman & Trube, 1985; Sturgess et al., 1985; Cook et al., 1988), leading to depolarization of the cell membrane and activation of voltage-operated Ca^{2+} channels (VOCCs) which mediate the influx of Ca^{2+} (Arkhammar et al., 1987; Gylfe, 1988a,b; Keahey et al., 1989). The subsequent increase in cytosolic [Ca^{2+}] is a critical signal in the induction of insulin secretion (Wollheim & Sharp, 1981; Arkhammar et al., 1987; Gylfe, 1988a,b).

Metabolism of fuel secretagogues, such as D-glucose, also induces hydrolysis of islet β-cell membrane phospholipids and the liberation of nonesterified arachidonate (Turk et al., 1986), which accumulates in amounts sufficient in principle to achieve concentration increments of 35–70 μM within β-cells (Wolf et al., 1986, 1991). Nonesterified arachidonate mobilizes intracellular Ca^{2+} (Wolf et al., 1986) and enhances Ca^{2+} influx into β-cell (Ramanadham et al., 1992; Wolf et al., 1991; Metz et al., 1987). The latter effect of arachidonate may reflect its ability to reduce the threshold rise in membrane potential required to activate VOCCs (Vacher et al., 1989) and may explain the amplification of depolarization-induced insulin secretion by arachidonate (Wolf et al., 1991).

The liberation of nonesterified arachidonate is mediated by phospholipases A_2 (PLA_2) which are ubiquitous lipolytic enzymes that catalyze hydrolysis of *sn*-2 fatty acyl residues from glycerophospholipid substrates to yield free fatty acid and a 2-lysophospholipid. Most recognized PLA_2 enzymes require Ca^{2+} or ligand–receptor–G-protein interactions for activation (Dennis et al., 1991; Leslie et al., 1991; Takayama et al., 1991; Clark et al., 1991; Burch et al., 1986). Since glucose-induced hydrolysis of arachidonate from β-cell phospholipids requires that glucose be metabolized within β-cells (Turk et al., 1992) but does not require Ca^{2+} influx (Wolf et al., 1991) and because ATP has been proposed as a second messenger of glucose metabolism in β-cells (Cook & Hales, 1984; Cook et al., 1988; Misler et al., 1986), the sensitivity of PLA_2 activities expressed in islets to Ca^{2+} and ATP has recently been examined.

The dominant islet PLA_2 activity was found to be fully active in the absence of free Ca^{2+} and to be activated by ATP, leading to its designation as an ATP-stimulatable Ca^{2+}-independent phospholipase A_2 (ASCI-PLA_2) (Gross et al., 1993; Ramanadham et al., 1993c). Other properties of the islet ASCI-PLA_2 activity included a strong preference for plasmalogen over diacylphospholipid substrates and susceptibility to inhibition by HELSS (Hazen et al., 1991; Ramanadham et al., 1993c).

The Ca^{2+}-independence of islet ASCI-PLA_2, although unusual among PLA_2 enzymes, is not unprecedented. A subgroup of PLA_2 enzymes have been identified in heart, brain, lung and other cells that are active in the absence of Ca^{2+} (Ross et al., 1985; Wolf & Gross, 1985; Ballou et al., 1986; Nijssen et al., 1986; Husebye & Flatmark, 1987; Pierik et al., 1988; Gassama-Diagne et al., 1989; Hazen et al., 1990; Hirashima et al., 1992; Yost et al., 1992). The best characterized of these is the myocardial enzyme which, similar to the islet enzyme, prefers plasmalogen substrates, is inhibited by HELSS, and is activated by ATP (Wolf & Gross, 1985; Hazen & Gross, 1991). The preference of the myocardial enzyme for plasmalogen substrates is observed even when the enzyme is presented with a binary mixture comprised of only 10 mol % plasmenylcholine in phosphatidylcholine bilayers (Hazen et al., 1990). The myocardial enzyme is comprised of a large (350–400 kDa) regulatory polypeptide complex and a smaller (40 kDa) catalytic polypeptide (Hazen & Gross, 1991).

Evidence that islet ASCI-PLA_2 is closely linked to islet β-cell function includes the observations that inhibition of the islet enzyme with HELSS suppresses several glucose-induced events, including hydrolysis of arachidonate from islet membrane phospholipids, Ca^{2+} influx into β-cells, and insulin secretion (Ramanadham et al., 1993c). The inhibitory effect of HELSS appears to be specifically targeted toward the islet ASCI-PLA_2, and the compound does not inhibit islet phosphoinositide–phospholipase C or glucose oxidation (Hazen et al., 1991; Ramanadham et al., 1993c).

An important objective is to achieve molecular characterization of the protein molecule responsible for islet ASCI-PLA_2 activity. Since only relatively small amounts of islets can be feasibly isolated at a given time, clonal pancreatic β-cell lines were considered an attractive alternative because they can be cultured in relatively large quantities and because they represent a homogeneous population of β-cells that is not contaminated with other cell populations (e.g., α-cells) contained in intact islets. One such candidate was the RIN-m5f cell line (Chick et al., 1977). However, these cells are unresponsive to glucose (Bhathena et al., 1983; Gylfe et al., 1983; Halban et al., 1983; Praz et al., 1983) and also did not express an ASCI-PLA_2 activity (Gross et al., 1993). In

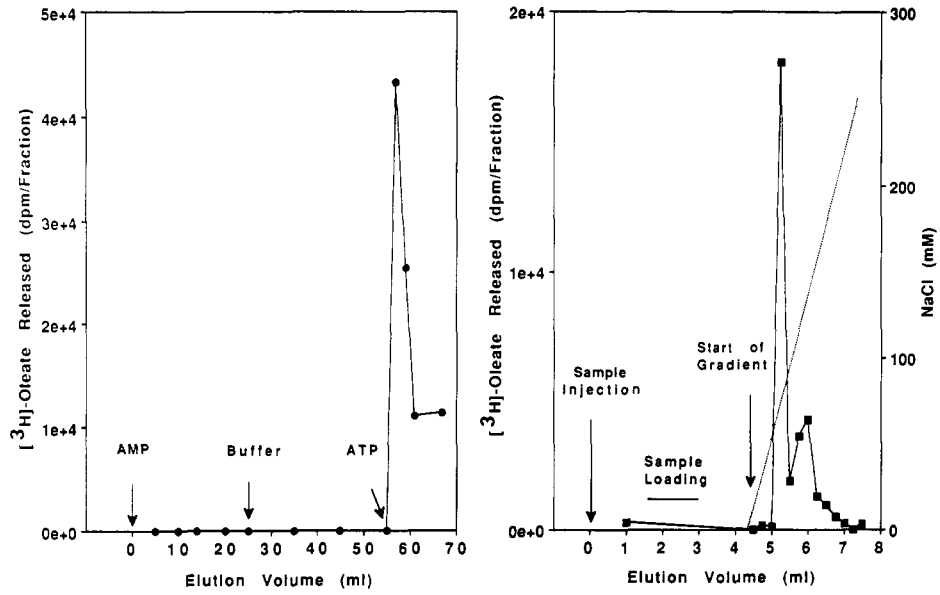

FIGURE 9: Chromatographic characterization of rat pancreatic islet ASCI-PLA_2. Pancreatic islets were isolated from 60 rats, and the cytosol was prepared, dialyzed, and analyzed by sequential ATP-affinity and Mono Q anion-exchange chromatography, as described in Figure 7. The ASCI-PLA_2 activity in column fractions was assayed as described in Table 1. Left panel: ATP-affinity chromatographic analysis of rat ASCI-PLA_2. Right panel: Mono Q analysis of rat ASCI-PLA_2: [- - -, NaCl gradient; closed symbols, ASCI-PLA_2 activity (dpm)].

contrast to the RIN-m5f cells, HIT cells are similar to islets in many crucial respects. They secrete insulin in response to glucose (Hill & Boyd, 1985; Ashcroft et al., 1986; Lambert et al., 1986; Meglasson et al., 1987; Zhang et al., 1989). Glucose metabolism in the HIT cells is similar to that in islet β-cells (Shimizu et al., 1988) and leads to increases in cytosolic [Ca^{2+}] (Hughes & Ashcroft, 1988; Meats et al., 1989; Hughes et al., 1990; Regazzi et al., 1990). HIT cells also express K_{ATP} channels (Kunze et al., 1987; Ashcroft et al., 1988; Aguilar-Bryan et al., 1992) as do pancreatic islet β-cells and cardiac myocytes (Noma, 1983; Cook & Hales, 1984; Weiss & Lamp, 1987). These properties of HIT cells suggest that they are an appropriate model for the study of β-cell function.

In the studies described here, HIT cells were found to express a Ca^{2+}-independent PLA_2 activity which preferred plasmalogen substrates containing *sn*-2 arachidonate residues. The HIT cells were also found to contain substantial endogenous levels of plasmenylethanolamine molecular species containing arachidonate in the *sn*-2 position. The HIT cell Ca^{2+}-independent PLA_2 activity was inhibited by the plasmalogen analog HELSS in a concentration-dependent manner, and HELSS suppressed both glucose-induced eicosanoid release and insulin secretion from HIT cells.

The HIT cell Ca^{2+}-independent PLA_2 activity was also selectively activated by ATP, indicating that an ASCI-PLA_2 activity is expressed in HIT cells. This effect of ATP was mimicked by the nonhydrolyzable ATP analog AMP-PCP, suggesting that an intermediary kinase is not involved in mediating the effect of ATP (Eckstein, 1983; Lohner et al., 1984; Nishizuka, 1986). GTP did not exert a similar effect, and this finding does not implicate, but does not exclude, G-protein-mediated events (Stryer & Bourne, 1986; Gilman, 1987; Birnbaumer et al., 1990) in ASCI-PLA_2 activation. ATP, in addition to activating the HIT cell ASCI-PLA_2, also attenuated thermal denaturation of the enzyme. A further reflection of the specific interaction of ATP with ASCI-PLA_2 is the quantitative adsorption of the enzyme to an ATP-affinity matrix and its selective desorption by ATP.

There has been intense interest in the possibility that ATP serves as a second messenger of glucose metabolism in islets (Cook & Hales, 1984; Cook et al., 1988; Misler et al., 1986). Uncertainty about the precise role of ATP in modulating the activity of metabolic response elements in the β-cell, such as the K_{ATP} channel, arises from the observations that resting islet and HIT cell ATP concentrations are in the range of 1–2 mM (Meglasson et al., 1989; Niki et al., 1989; Ohta et al., 1990, 1991, 1992, 1993; Ghosh et al., 1991), but inactivation of the K_{ATP} channel by ATP in islets (Cook & Hales, 1984) and HIT cells (Dunne et al., 1986) can occur with micromolar concentrations of ATP. In addition, relatively small increases in intracellular islet ATP concentrations have been observed following acute stimulation with glucose (Ghosh et al., 1991), although a doubling of islet ATP concentration after a 30-min stimulation with glucose has been reported (Thanakitcharu et al., 1991; Fadda et al., 1992; Zhou et al., 1992).

Several potential explanations have been advanced to account for the discrepancy between effective and resting concentrations of ATP with respect to K_{ATP} channel activity. These include the possibility that the submembrane ATP concentration is considerably lower than the average intracellular concentrations (Niki et al., 1989) or that the K_{ATP} channel response is modulated by intracellular concentrations of ADP (Kakei et al., 1986), which may change more substantially in glucose-stimulated islets than the concentrations of ATP (Meglasson et al., 1989; Ohta et al., 1990, 1991, 1992, 1993). Similar considerations may apply to the possibility that β-cell ASCI-PLA_2 activity is regulated by ATP. In the presence of Mg^{2+}, activation of the HIT cell enzyme by ATP occurred at ATP concentrations which approximate those found in intact islets, and ADP produced a concentration-dependent inhibition of ATP-stimulated enzymatic activity. These results suggest that ADP may influence the interaction of ASCI-PLA_2 with ATP. Although interaction with ATP and ADP are clearly biochemical properties of β-cell ASCI-PLA_2, the possibility that physiologic regulation of the activity of the enzyme is also influenced by other signals derived from the metabolism of glucose must be entertained.

In this regard, it is of interest that the analogous myocardial enzyme was recently demonstrated to exist as a complex with an isoform of the glycolytic enzyme phosphofructokinase (PFK) and that the sensitivity of the enzyme to ATP is

conferred as a result of this association (Hazen and Gross, 1993). The possibility that a similar complex exists in β-cells is of great interest since the concentrations of a number of substances which interact with PFK, such as fructose 6-phosphate and fructose 1,6-bisphosphate, change rather substantially upon stimulation of islets with glucose (Meglasson & Matschinsky, 1986). Studies to elucidate the potential interaction of islet PFK with islet ASCI-PLA_2 activity are currently underway.

In summary, the data presented herein represent the first demonstration of expression by a clonal insulin-secreting line of β-cells of an ATP-stimulatable Ca^{2+}-independent PLA_2 whose properties are similar to the analogous islet ASCI-PLA_2. This study also includes the first demonstration that HIT cells contain substantial endogenous pools of arachidonate-containing plasmenylethanolamine molecular species. These findings suggest that expression of ASCI-PLA_2 and of its preferred plasmalogen substrates may be a general property of insulin-secreting β-cells and that HIT cells may be an useful model of the pancreatic islet β-cell for further study of ASCI-PLA_2 and the events linking fuel secretagogue-induced insulin secretion and membrane phospholipid hydrolysis.

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