Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1411–1415, February 1990
Cell Biology

Activation of phosphatidylinositol 3-kinase by insulin

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Communicated by Konrad Bloch, October 23, 1989

ABSTRACT **Insulin action appears to require the protein-tyrosine kinase domain of the β subunit of the insulin receptor. Despite this, the identities and biochemical functions of the cellular targets of this tyrosine kinase are unknown. A phosphatidylinositol 3-kinase (PI 3-kinase) that phosphorylates the D-3 position of the inositol ring associates with several protein-tyrosine kinases. Here we report that PI 3-kinase activity is immunoprecipitated from insulin-stimulated CHO cells by anti-phosphotyrosine and anti-insulin receptor antibodies. Insulin as low as 0.3 nM increased immunoprecipitable PI 3-kinase activity within 1 min. Increases in activity were much greater in CHO cells expressing the human insulin receptor (100,000 receptors per cell) than in control CHO cells (2000 receptors per cell). During insulin stimulation, various lipid products of the PI 3-kinase either appeared or increased in quantity in intact cells, suggesting that the appearance of immunoprecipitable PI 3-kinase reflects an increase in its activity *in vivo*. These results indicate that insulin at physiological concentrations regulates the PI 3-kinase and suggest that this regulation involves a physical association between the insulin receptor and the PI 3-kinase and tyrosyl phosphorylation.**

The insulin receptor is a tetrameric glycoprotein composed of two α and two β subunits. The β subunit is a protein-tyrosine kinase that undergoes autophosphorylation upon insulin binding to the α subunit (1). Autophosphorylation induces a conformational change in the β subunit and activates the tyrosyl kinase (2, 3). Experiments involving removal of autophosphorylation sites and inactivation of the ATP binding site indicate that the kinase domain is required for insulin action (4–7). Transmission of the insulin signal may occur through a cascade of protein phosphorylation (8) and the generation of second messengers (9). Despite considerable work in this area, the cellular targets of the insulin receptor and the precise identities of the second messengers that are critical for transmitting its signals have yet to be elucidated.

A phosphatidylinositol (PtdIns) kinase has been described that specifically associates with certain protein-tyrosine kinases (10). This enzyme phosphorylates the D-3 position of the inositol ring of PtdIns to produce phosphatidylinositol 3-phosphate (PtdIns3*P*) (11). This enzyme termed PtdIns 3-kinase was shown to physically associate with the polyomavirus middle-size tumor antigen–pp60$^{c\text{-}src}$ complex (12) and with the platelet-derived growth factor (PDGF) receptor (10). An 85-kDa protein that specifically associates with both the middle-size tumor antigen–pp60$^{c\text{-}src}$ complex and the PDGF receptor and that is phosphorylated on tyrosine *in vivo* has been shown to copurify with this enzyme (10, 13). The importance of the PtdIns 3-kinase for transmitting growth signals has been supported by studies of mutants of the polyoma middle-size tumor antigen gene (12), the v-*src* gene (14), and the PDGF receptor gene (15).

In addition to phosphorylation of PtdIns, this enzyme phosphorylates phosphatidylinositol 4-phosphate (PtdIns4*P*) and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] to form phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2] and phosphatidylinositol trisphosphate {PtdInsP_3, probably phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3]}, respectively (16). These PtdIns phosphates are not in the pathway for hormone-stimulated inositol 1,4,5-trisphosphate production and are not substrates for PtdIns-specific phospholipase C enzymes (17). Identical or related compounds are produced in smooth muscle cells during PDGF stimulation (16) and in fibroblasts during polyoma middle-sized tumor antigen transformation (L. A. Serunian, K. R. Auger, T. M. Roberts, and L.C.C., unpublished work). Furthermore, PtdInsP_3 is produced in fMet-Leu-Phe-stimulated neutrophils (19).

We investigated the possibility that the PtdIns 3-kinase is activated by the insulin receptor because insulin and PDGF cause a number of common responses in cells that express both receptors (20). Here we show that the PtdIns 3-kinase specifically associates with the insulin receptor upon insulin addition to intact cells. We also show that the lipid products of this enzyme are elevated in insulin-stimulated cells.

EXPERIMENTAL PROCEDURES

Cell Culture. CHO cells transfected with the neomycin-resistance gene (CHO/Neo) or the neomycin-resistance gene plus the gene for the human insulin receptor (CHO/IR) were prepared as described (21). Cells were cultured in Ham's F12 medium containing 10% (vol/vol) fetal bovine serum and Geneticin (G418;400 μg/ml). Cells were grown to ≈90% confluence in 100-mm dishes and then incubated overnight in Ham's F12 medium containing 0.5% albumin (quiescing medium). The CHO/Neo and the CHO/IR cells express 2000 and 100,000 insulin receptors per cell, respectively, as judged by insulin binding (21).

Assay of PtdIns 3-Kinase in Immunoprecipitates. After incubation with insulin or another agonist, the cells were lysed at 4°C in buffer containing 20 mM Tris·HCl, (pH 8.1), 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10% (vol/vol) glycerol, Nonidet P-40; 150 μM vandate; 1 mM phenylmethylsulfonyl fluoride, and aprotinin (10 μg/ml). The lysate was incubated with either anti-phosphotyrosine {anti-Tyr(*P*) [hybridoma supernatant (100 μl/ml) of lysate from T. Roberts (8)]} or anti-insulin receptor antibody {anti-960 [20 μg/ml of lysate; antibody raised against a synthetic peptide that corresponds to the region surrounding Tyr-960 of the insulin receptor (22)]} for 1 hr and then for 1.5 hr with protein A-Sepharose (4 μg/ml of lysate). The immunoprecipitates were washed thrice with isotonic phosphate-buffered saline (PBS)/1% Nonidet P-40, twice with 0.5 M LiCl/100 mM Tris·HCl, pH 7.6, and twice

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Abbreviations: PDGF, platelet-derived growth factor; IGF-I, insulin-like growth factor I; PtdIns, phosphatidylinositol; PtdIns*P*, PtdIns3*P*, and PtdIns4*P*, phosphatidylinositol phosphate, phosphatidylinositol 3-phosphate, and phosphatidylinositol 4-phosphate, respectively; PtdInsP_2, PtdIns(3,4)P_2, and PtdIns(4,5)P_2, phosphatidylinositol bisphosphate, phosphatidylinositol 3,4-bisphosphate, and phosphatidylinositol 4,5-bisphosphate, respectively; PtdInsP_3 and PtdIns(3,4,5)P_3, phosphatidylinositol trisphosphate and phosphatidylinositol 3,4,5-trisphosphate, respectively.

with 10 mM Tris·HCl, pH 7.4/100 mM NaCl/1 mM EDTA. The lipid kinase assay and lipid extraction were performed directly in the beads as described (16, 23). Phospholipids contained in the organic phase were separated by TLC either in chloroform/methanol/water/ammonia [60:47:11.3:2 (vol/vol)] or in 1-propanol/2 M acetic acid 65:35 (vol/vol). The latter solvent system is used for better separation of phosphatidylinositol phosphate (PtdIns*P*), phosphatidylinositol bisphosphate (PtdInsP_2), and PtdInsP_3. For definitive identification of phosphatidylinositol phosphates detected on the TLC plate, the radioactive spots were extracted, deacylated, and subjected to HPLC analysis (see below).

Metabolic Labeling. For labeling with [^{35}S]methionine, cells were cultured for 16 hr in 4 ml of serum-free RPMI select medium containing 3% of the usual concentration of unlabeled methionine, Trans^{35}S-label methionine (200–300 μCi/ml; 1 Ci = 37 GBq; ICN), and 2% (wt/vol) albumin. After the labeling period, insulin (670 nM) [GIBCO] was added to the cells. After 10 min the cells were lysed and polypeptides were immunoprecipitated with either anti-Tyr(*P*) antibody or anti-960 antibody as described above. The immunoprecipitated proteins were eluted with SDS sample buffer (18) and analyzed by SDS/PAGE.

For labeling with [^{32}P]orthophosphate (NEN), subconfluent cells were cultured in fetal bovine serum-free medium for 16 hr and then incubated in phosphate-free Dulbecco's modified Eagle's medium containing 0.5% albumin for 15 min. Carrier-free [^{32}P]orthophosphate (100 μCi/ml) was added. After 2.5 hr at 37°C, insulin was added. Periodically, the cells were washed with ice-cold PBS, lysed in 1 M HCl/methanol, 1:1 (vol/vol), and extracted in chloroform. Lipid-containing phases were deacylated and analyzed by HPLC (16, 23).

RESULTS

Activation of PtdIns Kinase by Insulin. Upon addition of insulin to CHO cells, a marked increase was observed in anti-Tyr(*P*)-immunoprecipitable PtdIns kinase activity (Fig. 1). The response was detectable in CHO/Neo cells, which have ≈2000 insulin receptors per cell (22), but the response was much greater in CHO/IR (≈100,000 receptors per cell; ref. 22). The increase in PtdIns 3-kinase appeared to plateau at 1.3 nM insulin, although a supraphysiological concentration of insulin (670 nM) caused an additional increase in both cell lines. In a separate experiment, 0.3 nM insulin was shown to be nearly as effective as 1.3 nM insulin in CHO/IR cells (see below). Insulin-like growth factor I (IGF-I) was only ≈25% as effect as insulin in stimulating the immunoprecipitable PtdIns kinase activity when used at 1.3 nM (data not shown). Thus these findings indicate that the major effect of insulin in CHO/IR cells is mediated by the insulin receptor rather than the IGF-I receptor. The assays shown in Fig. 1 utilized both PtdIns and PtdIns4*P* as substrates, suggesting that insulin activates a PtdIns and a PtdIns*P* kinase activity by interacting with its own receptor.

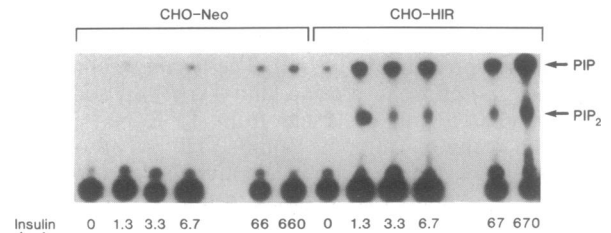

FIG. 1. Anti-Tyr(*P*)-immunoprecipited PtdIns kinase activity from insulin-stimulated cells. Subconfluent CHO/Neo cells and CHO/IR cells (CHO-HIR) were stimulated as indicated with insulin (nM) for 10 min. The cells were lysed and incubated with anti-Tyr(*P*) antibody. A mixture of PtdIns and PtdIns4*P* was added to the immunoprecipitate and the reaction was started by addition of [γ-^{32}P]ATP. The products were extracted with chloroform/methanol and separated by TLC. An autoradiogram is shown. PtdIns4*P* (PtdIns*P*) and PtdIns(4,5)P_2 (PtdInsP_2) standards are indicated by arrows. The radioactivity at the origin is due to [^{32}P]ATP and other water-soluble ^{32}P-labeled materials that were not completely extracted from the chloroform layer.

Immunoprecipitation of [^{35}S]Methionine-Labeled Proteins in CHO/IR Cells by Anti-Tyr(*P*) and Anti-Insulin Receptor Antibodies. The ability of the anti-Tyr(*P*) antibody to immunoprecipitate the insulin receptor and associated proteins was investigated by using [^{35}S]methionine-labeled CHO/IR cells (Fig. 2). The anti-Tyr(*P*) antibody failed to cause significant immunoprecipitation of proteins from cells not stimulated with insulin. Two major proteins whose molecular masses correspond to those of the α and β chains of the insulin receptor (130 kDa and 95 kDa) were immunoprecipitated from insulin-stimulated cells (Fig. 2*A*). Some additional less-abundant proteins were also detected (see below).

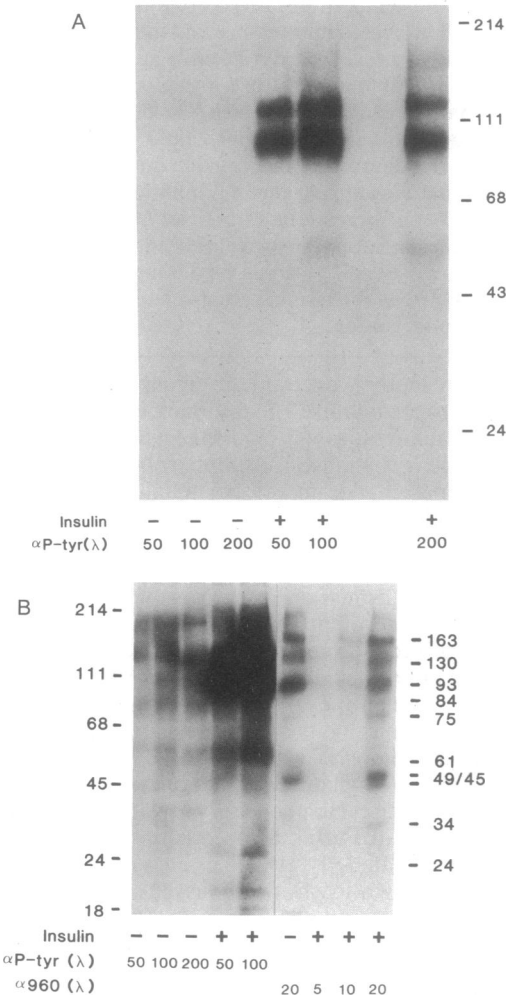

FIG. 2. Immunoprecipitation of [^{35}S]methionine-labeled polypeptides by anti-Tyr(*P*) (αP-tyr) and anti-insulin receptor (α960) antibodies. (*A*) Autoradiograph (7-hr exposure at −70°C) of an SDS/polyacrylamide gel of anti-Tyr(*P*)-immunoprecipitated protein. At this exposure the bands for the α (135 kDa) and β (95 kDa) subunits of the insulin receptor were only evident in cells stimulated by insulin. (*B*) Longer exposure of the gel in *A* (38-hr exposure at −70°C) showing the relative amounts of proteins precipitated by the anti-insulin receptor antibody and the anti-Tyr(*P*) antibody. The amount of insulin receptor in the anti-960 immunoprecipitates was not affected by the presence or absence of insulin. The apparent molecular masses (kDa) of some of the major proteins are indicated. +, Insulin added (670 nM for 10 min); −, insulin absent.

Parallel experiments were performed using an antibody raised against a peptide from the cytosolic domain of the insulin receptor (anti-960 antibody). With this antibody, the insulin receptor α (130 kDa) and β (95 kDa) subunits and a 163-kDa protein (probably the insulin receptor precursor) were detected (Fig. 2*B*). At the concentrations of antibodies used in these experiments, the anti-Tyr(*P*) antibody was much more efficient at immunoprecipitating the receptor (Fig. 2*B* shows the results of identical exposures of parallel experiments). As expected with anti-insulin receptor antibody, the abundance of receptor in the immunoprecipitate was not affected by addition of insulin to the cells, since the binding of this antibody is not dependent on the phosphorylation state of the receptor. In some anti-insulin receptor immunoprecipitates an 84-kDa protein and several other peptides were sometimes detected in cells stimulated with insulin. An 84-kDa protein was also phosphorylated when anti-Tyr(*P*) immunoprecipitates from insulin-stimulated cells were incubated with [γ-^{32}P]ATP (data not shown). Because of the proximity of this band to the β subunit of the insulin receptor, its presence may sometimes be difficult to detect. Whether the 84-kDa peptide or any of the other proteins detected by the methionine labeling is the PtdIns 3-kinase remains to be determined.

Immunoprecipitation of PtdIns Kinase by Anti-Insulin Receptor Antibody. Evidence that the PtdIns kinase directly associates with the insulin receptor was provided by the detection of this activity in anti-insulin receptor immunoprecipitates (Fig. 3). PtdIns kinase activity was detected in the anti-960 antibody immunoprecipitates from insulin-treated cells (Fig. 3). The activity was nearly absent in immunoprecipitates from unstimulated cells even though the same amount of receptors was recovered. The total activity recovered was less than that observed in a parallel experiment using the anti-Tyr(*P*) antibody (Fig. 3). Both PtdIns conversion to a monophosphorylated PtdIns and PtdIns-4*P* conversion to a PtdInsP_2 species were detected with the anti-960 immunoprecipitate.

To address whether PtdIns 3-kinase is an intrinsic activity of the insulin receptor, cell lysates from insulin-stimulated cells were sequentially immunoprecipitated with anti-insulin receptor antibody and anti-Tyr(*P*) antibody. The PtdIns 3-kinase activity in the two immunoprecipitates was then compared. Anti-insulin receptor antibody precipitated ≈5 times more receptor than did the anti-Tyr(*P*) antibody (as judged by methionine-labeled protein). However, the anti-Tyr(*P*) immunoprecipitates had ≈10 times as much PtdIns 3-kinase activity (data not shown). Thus, although some of the PtdIns 3-kinase associated with the insulin receptor, most of the anti-Tyr(*P*) precipitable enzyme did not.

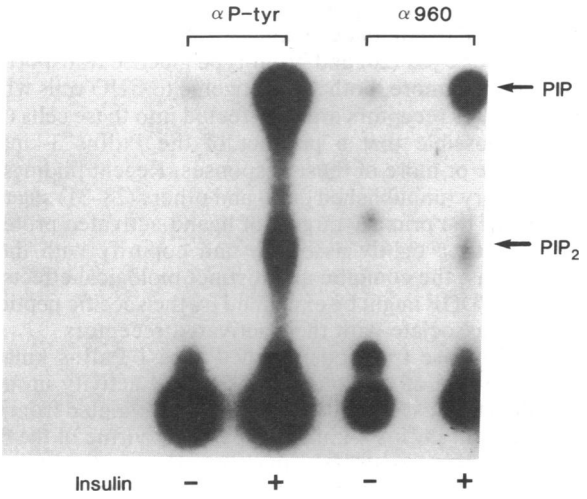

FIG. 3. Immunoprecipitation of PtdIns kinase by anti-insulin receptor antibody (α 960) and anti-Tyr(*P*) antibody (αP-tyr). Immunoprecipitation of PtdIns kinase activity in insulin-stimulated cells by anti-960 antibody was confirmed in three additional experiments. +, Insulin added; −, insulin absent. PIP, PtdIns*P*; PIP$_2$, PtdInsP_2.

Identification of the Lipid Products of the PtdIns Kinase. Since the lipid kinase activity that associated with the PDGF receptor was shown to phosphorylate PtdIns, PtdIns4*P*, and PtdIns(4,5)P_2 to form three lipid species (16), we investigated the ability of the insulin-receptor-associated activity to catalyze these reactions. When all three lipids were added at equal concentrations to anti-Tyr(*P*) immunoprecipitates, three products with TLC migration properties consistent with the structures PtdIns*P*, PtdInsP_2, and PtdInsP_3 were formed (Fig. 4). Under these conditions, PtdIns was phosphorylated somewhat more efficiently than PtdIns4*P* or PtdIns(4,5)P_2.

Each of these lipids was extracted from the thin layer plate, deacylated, and analyzed by HPLC (Fig. 4). The deacylated PtdIns*P* (Fig. 4*A*), PtdInsP_2 (Fig. 4*B*), and PtdInsP_3 (Fig. 4*C*) had identical elution profiles as deacylated PtdIns3*P*, PtdIns(3,4)P_2, and PtdInsP_3 produced in anti-Tyr(*P*) immunoprecipitates of PDGF-stimulated cells, respectively. All three of these products clearly separated from the deacylation products of PtdIns4*P* and PtdIns(4,5)P_2. Identical results were obtained using lipids formed by anti-960 antibody immunoprecipitates (data not shown). Thus, the products formed by the insulin-receptor-associated PtdIns kinase appear to be the same as those formed by the PDGF-receptor-associated PtdIns kinase (16).

Onset and Duration of Insulin Action. In CHO/IR cells stimulated by insulin, PtdIns 3-kinase activity in the anti-Tyr(*P*) immunoprecipitate was maximal within 1 min (Fig. 5*A*). The ability of the immunoprecipitates to phosphorylate all three lipids did not significantly change between 1 and 20 min after insulin addition. However, a significant decrease in the ability to phosphorylate all three lipids was observed at 30 and 60 min after insulin addition. The data presented in Fig. 5*A* are from a single experiment and are representative of four experiments. However, some variability in the relative utilization of the three substrates was found.

Insulin Stimulates Production of Phosphatidylinositol Phosphates in Intact Cells. Lipids that comigrate with the products of the PtdIns 3-kinase formed *in vitro* are produced in intact cells in response to insulin. CHO/IR cells were incubated with [^{32}P]orthophosphate for 2.5 hr and then exposed to insulin prior to lipid extraction. The extracted lipids were deacylated and separated on anion-exchange HPLC (see Fig. 4). A ^{32}P-labeled compound that comigrated with deacylated PtdIns3*P* was present in samples from both the insulin-stimulated and unstimulated cells. This compound was ≈3% as abundant as PtdIns4*P*, and its level did not increase upon insulin stimulation (data not shown). In contrast, ^{32}P-labeled compounds that comigrated with the deacylation products of PtdIns(3,4)P_2 and PtdInsP_3 were marginally detectable in unstimulated cells and increased markedly after 1 min of stimulation by insulin (Fig. 5*B*). Radiolabeled PtdIns4*P* and PtdIns(4,5)P_2 were abundant in samples from unstimulated cells and were not increased by insulin. CHO cells were also labeled with [^{3}H]inositol and the deacylated PtdIns3*P* peak was confirmed with this label. Thus, consistent with the detection of the PtdIns 3-kinase activity in insulin receptor immunoprecipitates, the lipid products of this enzyme appear to increase upon stimulation of intact cells with insulin.

DISCUSSION

The results indicate that at physiological concentrations insulin rapidly activates PtdIns 3-kinase in CHO/IR cells. The activation appears to be due to a physical association of the PtdIns 3-kinase with the insulin receptor since this enzyme was immunoprecipitated by an anti-insulin receptor

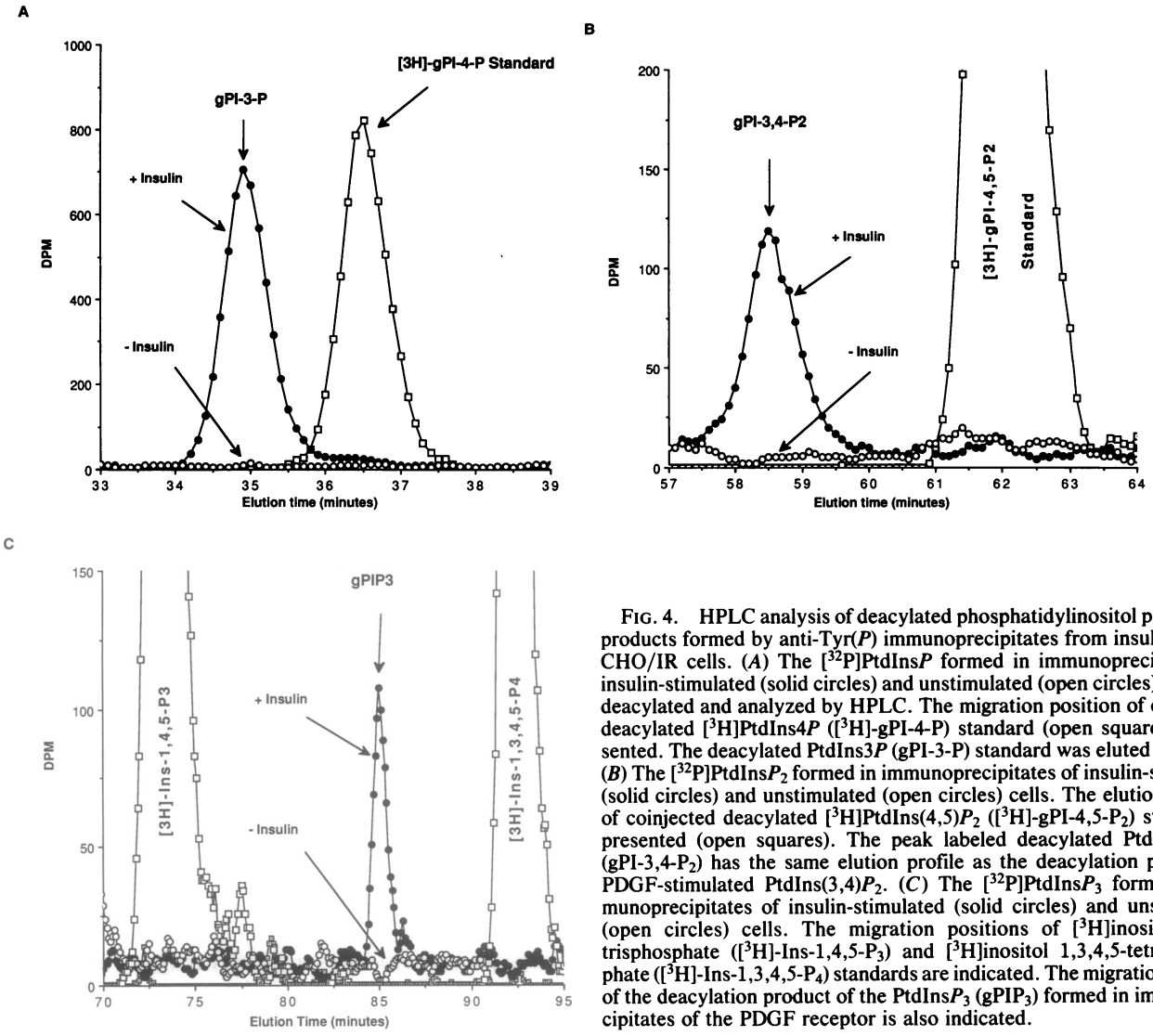

FIG. 4. HPLC analysis of deacylated phosphatidylinositol phosphates products formed by anti-Tyr(*P*) immunoprecipitates from insulin-treated CHO/IR cells. (*A*) The [^{32}P]PtdIns*P* formed in immunoprecipitates of insulin-stimulated (solid circles) and unstimulated (open circles) cells was deacylated and analyzed by HPLC. The migration position of coinjected deacylated [^{3}H]PtdIns4*P* ([^{3}H]-gPI-4-P) standard (open squares) is presented. The deacylated PtdIns3*P* (gPI-3-P) standard was eluted at 35 min. (*B*) The [^{32}P]PtdInsP_2 formed in immunoprecipitates of insulin-stimulated (solid circles) and unstimulated (open circles) cells. The elution position of coinjected deacylated [^{3}H]PtdIns(4,5)P_2 ([^{3}H]-gPI-4,5-P_2) standard is presented (open squares). The peak labeled deacylated PtdIns(3,4)P_2 (gPI-3,4-P_2) has the same elution profile as the deacylation product of PDGF-stimulated PtdIns(3,4)P_2. (*C*) The [^{32}P]PtdInsP_3 formed in immunoprecipitates of insulin-stimulated (solid circles) and unstimulated (open circles) cells. The migration positions of [^{3}H]inositol 1,4,5-trisphosphate ([^{3}H]-Ins-1,4,5-P_3) and [^{3}H]inositol 1,3,4,5-tetrakisphosphate ([^{3}H]-Ins-1,3,4,5-P_4) standards are indicated. The migration position of the deacylation product of the PtdInsP_3 (gPIP_3) formed in immunoprecipitates of the PDGF receptor is also indicated.

antibody. Involvement of the insulin receptor rather than the IGF-I receptor is also suggested by the finding that the response was greater in CHO/IR cells than in CHO/Neo cells: on a per cell basis, the CHO/IR cells possess ≈100,000 insulin receptors compared to 2000–3000 in CHO/Neo, whereas both cells possess 5000–10,000 IGF-I receptors (24, 25). Finally, insulin was more effective than IGF-I in stimulating the PtdIns 3-kinase at physiological concentration. At these levels insulin has been shown to stimulate mitogenesis in CHO/IR cells (22).

The mechanism by which the PtdIns 3-kinase activity appears in immunoprecipitates of the insulin receptor in response to insulin stimulation is still not clear. Perhaps a latent form of this enzyme is associated with unstimulated insulin receptors and becomes activated in response to insulin addition through tyrosyl phosphorylation. However, it is more likely that insulin binds to its receptor and subsequently autophosphorylation induces a conformational change in the cytosolic domain that allows the PtdIns 3-kinase and perhaps other proteins to bind. These proteins might then be targets for the tyrosyl kinase activity. Consistent with this hypothesis, we detected a number of low-abundance proteins that coimmunoprecipitated with the insulin receptor from cells stimulated with insulin but that failed to coimmunoprecipitate with the receptor from unstimulated cells (Fig. 2*B*).

The ability of both the insulin receptor and the PDGF receptor to bind to and activate the PtdIns 3-kinase suggests that products of this enzyme may mediate cellular responses common to these two growth factors. For example, both insulin and PDGF stimulate S6 kinase activity (26) and induce the mRNA for c-*fos* (20) and brain-type glucose transporters (27, 37). Furthermore, both are mitogenic to CHO cells when their respective receptors are transfected into these cells (15, 21). It is possible that a product of the PtdIns 3-kinase mediates one or more of these responses. Recent findings in this laboratory (unpublished data) and others (28–31) suggest that some of the primary targets of ligand-activated protein-tyrosine kinases tightly associate and copurify with these kinases. Thus, the common and distinct biological effects of insulin and PDGF might be explained by the specific peptides that tightly associate with their activated receptors.

PtdIns 3-kinase (previously called type I PtdIns kinase) comprises 3–8% of the total PtdIns kinase activity in most mammalian tissues and cells. It can be differentiated from the more abundant PtdIns 4-kinase (type II) by virtue of the fact that it is almost completely inactive when substrates are presented in detergent micelles: the PtdIns 4-kinase preferentially utilizes detergent-solubilized PtdIns (32). Two other laboratories have reported finding a PtdIns kinase activity associated with (33, 34) but separable from (35), the insulin receptor. Because of the presence of detergent in the assay (32), it is likely that PtdIns 4-kinase activity was measured in

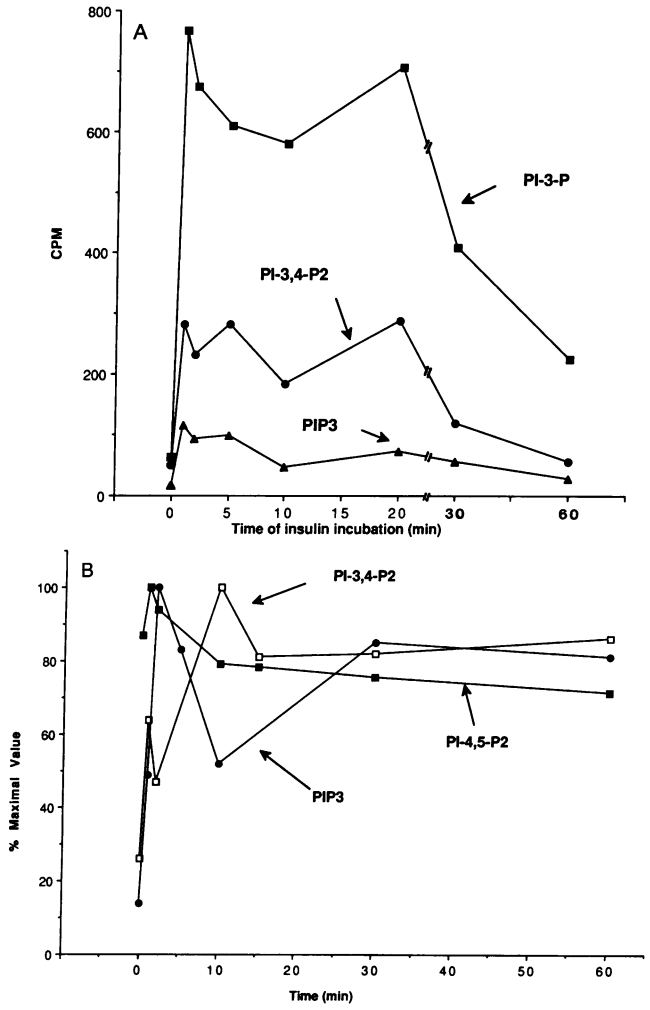

FIG. 5. Onset and duration of insulin activation of PtdIns 3-kinase in CHO/IR cells. (*A*) Immunoprecipitable lipid kinase activity from insulin-stimulated cells. Insulin (670 nM) was added to intact serum-starved CHO/IR cells for the times indicated. Anti-Tyr(*P*) immunoprecipitates from lysed cells were incubated with PtdIns/PtdIns4*P*/PtdIns(4,5)P_2/[γ-^{32}P]ATP. The quantity of ^{32}P incorporated into PtdIns3*P* (PI-3-P), PtdIns(3,4)P_2 (PI-3,4-P_2), and PtdInsP_3 (PIP_3) is shown as cpm. (*B*) Levels of PtdIns(4,5)P_2 (PI-4,5-P_2), PtdIns(3,4)P_2 (PI-3,4-P_2), and PtdInsP_3 (PIP_3) in intact cells stimulated with insulin. Quiescent CHO/IR cells were incubated in phosphate-free Ham's F12 medium containing 0.5% insulin-free bovine serum albumin and [^{32}P]orthophosphate (100 μC/ml) for 2.5 hr. At time zero insulin (670 nM) was added. At the indicated times, the cells were harvested in 1 M HCl/methanol and the chloroform-extracted lipids were deacylated and analyzed by HPLC as described above. The data points are averages of two or three experiments except for the 2-min and 10-min time points, which are from a single experiment. The ^{32}P in the HPLC peaks that comigrated with the deacylation products of PtdIns(4,5)P_2, PtdIns(3,4)P_2, and PtdInsP_3 is plotted. All data were normalized to total cpm in each lipid at time zero. The 100% values for the three lipids are 26,000 dpm [PtdIns(4,5)P_2], 300 dpm [PtdIns(3,4)P_2], and 280 dpm (PtdInsP_3).

these studies. As a consequence, the relationship of these data and those of the present study is unclear.

In conclusion, data have been presented that link PtdIns 3-kinase activation to the mechanism of insulin action. The possibility that the products of this enzyme, in particular PtdIns(3,4)P_2 and PtdInsP_3, mediate some insulin responses warrants further investigation.

Note. While this manuscript was in preparation we became aware of an independent investigation by G. Endemann *et al.* (36) indicating that anti-Tyr(*P*) antibodies immunoprecipitate the PtdIns 3-kinase from insulin-stimulated cells.

We thank Lyuba Varticovski and Kurt Auger for their advice throughout the study and Suzannah Paiewonsky and Deborah DiMaggio for their assistance in typing the manuscript. We also thank Gerda Endemann and Richard Roth for giving us a rough draft of their manuscript describing immunoprecipitation of PtdIns 3-kinase by anti-Tyr(*P*) antibodies from insulin-stimulated cells (36). This research was supported by grants from the National Institutes of Health (GM 36624 and DK 39249 to L.C.C; DK 19514 and DK 39814 to N.B.R.; and DK 38712 to M.F.W.

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