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The regulatory mechanism of phosphatidylinositol 3-kinase by insulin in 3T3 L1 fibroblasts: Phosphorylation - independent activation of phosphatidylinositol 3-kinase

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Abbreviations: PI, phosphatidylinositol; PI3-kinase, phosphatidylinositol 3-kinase; IP, immunoprecipitation; IRS-1, insulin receptor substrate-1; GST, glutathione S-transferase; SH2, src homology 2; SH3, src homology 3; C, control; I, insulin stimulated; PY, phosphotyrosine; PS, phosphoserine

Abstract

Phosphatidylinositol (PI) 3-kinase plays an important role in transducing the signals of various growth factor receptors. However, the regulatory mechanism of PI3-kinase activity by these growth factor receptors is not completely understood. Therefore, we attempted to clarify the regulatory mechanism of PI3-kinase using insulin and 3T3 L1 fibroblasts. Our results showed that insulin stimulated PI3-kinase activity seven-fold and concomitantly phosphorylated a p85 subunit at the tyrosine residue. However, this tyrosine phosphorylation was not significant in the activation of PI3-kinase as the PI3-kinase pulled down by the overexpressed GST-p85 fusion protein showed as high an activity as the immunoprecipitated one. The p110 subunit was phosphorylated at both serine and tyrosine residues without insulin treatment. Since the phosphorylation state was not changed by insulin. The results suggested that phosphorylation of the p110 subunit does not control PI3-kinase activity. Finally, it was shown that the insulin receptor substrate-1 (IRS-1) binding to PI3-kinase was not sufficient for full activation because the amount of IRS-1 pulled down by the GST-p85 fusion protein reached almost maximum, after incubation with insulin-treated cell lysates for 20 min, whereas PI3-kinase activity reached its maximum only after incubation for 5 h. All results suggest that the phosphorylation of p85 subunit at tyrosine residues and phosphorylation of p110 subunit at tyrosine or serine residues are not functionally significant in the regulation of PI3-kinase activity. They also suggest that PI3-kinase is needed to bind to other protein(s) as well as the insulin receptor substrate-1 for full activation.

Keywords: GST-p85 fusion protein, Insulin, PI3-kinase, 3T3 L1 fibroblast

Introduction

Since phosphatidylinositol 3-kinase (PI3-kinase) was first identified as a lipid kinase associated with middle T antigen in polyoma virus (SV40) transformed cells (Whitman *et al*., 1985), it has been demonstrated that PI3-kinase is activated by several different receptor tyrosine kinases such as receptors of insulin, epidermal growth factor, platelet-derived growth factor, *etc*. Also, it has been demonstrated that when these receptors are activated after binding to their ligands, PI3-kinase plays many important roles in the transduction of signals for cell proliferation, metabolic effects of insulin, membrane ruffling, membrane trafficking, cell motility, and immune responses (Cheatham *et al*., 1994; Kotani *et al*., 1994; Brown *et al*., 1995; Camilli *et al*., 1996; Rahimi *et al*, 1996).

PI3-kinase phosphorylates phosphatidylinositol (PI), phosphatidylinositol-4-monophosphate (PI-4-P), and phosphatidylinositol-4,5-diphosphate (PI-4,5-P_2) on the D-3 position of the inositol ring (Whitman *et al*., 1988; Divecha and Irvin, 1995). PI3-kinase was initially found to consist of two subunits, the p85 regulatory subunit and the p110 catalytic subunit. The p85 subunit mediates binding of the p110 subunit to tyrosine phosphorylated receptor tyrosine kinases through the SH2 domain of the p85 subunit (Backer *et al*., 1992). The p110 subunit exists as a monomeric form or heterodimeric form associated with the p85 subunit. Although the functional significance of protein kinase activity remains to be determined, the p110 subunit exhibits dual substrate specificity *in vitro*: it phosphorylates the phosphoinositide substrate as well as protein substrates such as the p85 subunit and IRS-1 at serine residues (Dhand *et al*., 1994;

Lam *et al*., 1994). Recently, multiple isoforms of PI3-kinase were discovered both in the p85 and the p110 subunits. The p85 subunit has α and β isoforms, and the p110 subunit has α, β and γ isoforms. The identification of these multiple PI3-kinase isoforms suggests the potential signalling properties of D-3 phosphoinositides during the transduction of cellular responses by diverse stimuli (Escobedo *et al*., 1991; Hiles *et al*., 1992; Hu *et al*., 1993). Furthermore, the PI3-kinase γ is activated by G protein βγ subunits without coupling to the p85 subunit. This suggests that PI3-kinase γ has distinct signalling properties from those elicited by tyrosine kinase signaling complexes (Stephens *et al*., 1994; Stoyanov *et al*., 1995).

After receptor tyrosine kinases were stimulated by ligand binding, PI3-kinases translocate from cytosol to the phosphorylated receptors on the tyrosine residues and then phosphorylate their lipid substrates located on the plasma membrane. The conformational changes in the p85 subunit driven by SH2 domain binding to phosphorylated YXXM motifs on the IRS-1 have been suggested in the activation of PI3-kinase (Backer *et al*., 1992; Rordorf-Nikolie *et al*., 1995; Antonetti *et al*., 1996). Other mechanisms for activation of PI3-kinase by growth factors have been proposed, including the binding of Src family SH3 domains to the p85 in lymphocytes during antigen receptor signalling (Pleiman *et al*., 1994) and the binding of the $p21^{ras}$ to PI3-kinase through p110 (Rodriguez-Viciana *et al*., 1994). But, recently, it was reported that nonisoprenylated $p21^{ras}$-GTP binds to p110 but does not activate PI3-kinase *in vitro*, suggesting that the $p21^{ras}$ may facilitate membrane targeting of p110 (Yu *et al*., 1998). Also, it has been shown that although growth factors induce tyrosine phosphorylation of the p85 subunit, it is still obscure as to whether this phosphorylation activates PI3-kinase. The functional significance of the phosphorylation state of the p110 subunit also remains to be clarified because this subunit is phosphorylated on tyrosine residues by PDGF in NIH 3T3 cells but is not phosphorylated by middle-T antigen-pp60c-src complexes.

Since it has been established that insulin activates PI3-kinase, it was decided to use insulin and 3T3 L1 fibroblasts to clarify the regulatory mechanisms of PI3-kinase. To perform this work, we used overexpressed GST-p85 fusion protein in *E. coli*, and then examined how phosphorylation of p85 and p110 subunits or IRS-1 binding to PI3-kinase affect PI3-kinase activity, using immunoprecipitation by an anti-phosphotyrosine antibody in parallel with GST pull down by this fusion protein. In this paper, we show that phosphorylation of the p85 subunit on tyrosine residues and phosphorylation of the p110 subunit on tyrosine and serine residues do not have significant effects on PI3-kinase activity. We also show that IRS-1 binding to PI3-kinase is not sufficient for the full activation of PI3-kinase activity. All these results suggest that other protein(s) as well as IRS-1 are needed to bind to the p85 or p110 subunits for full activation of PI3-kinase.

Materials and Methods

Materials

Antibodies specific to phosphotyrosine and phosphoserine were purchased from Sigma Chemical Co. (St. Louis, MO). Antibodies against p85α, p110α(C-17) and IRS-1 (C-14) were purchased from Upstate Biochemical Inc. cDNA for p85α was kindly provided by Dr. Kasuga in Japan. [γ-^{32}P]-ATP was obtained from New England Nuclear (Boston, MA). Glutathione-Sepharose resins and glutathione were purchased from Phamacia (Uppsala, Sweden) and from Boehringer Mannheim (Germany), respectively. Donor calf serum and Dulbeco's Modified Eagle's Medium (DMEM) were purchased from Gibco BRL (Buckinghamshire, UK). Silica gel 60 TLC plate was obtained from Merck. Protein A-Sepharose, Sepharose and other reagents were products of Sigma Chemical Co. ECL kit was from Amhersham

Cell culture

3T3 L1 fibroblasts were grown in DMEM containing 2 mM glutamine supplemented with 10% donor calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B under 5% CO_2 donditions in a humidified air at 37˚C. When cells were grown to near confluence, they were released by trypsin-EDTA treatment and then plated again in 100-mm petri dishes. To induce quiescence of the cells, they were exposed to serum free DMEM for 24 h prior to insulin treatment. To stimulate the cells, they were incubated with insulin at a 1 μM concentration for 5 min at 37˚C in a CO_2 incubator.

PI3-kinase assay

PI3-kinase activity was measured either by immunoprecipitation with an anti-phosphotyrosine antibody or by a GST-p85 fusion protein. The immunoprecipitation was done as follows. After washing twice with ice-cold PBS, control and insulin-treated cells were solubilized by incubation for 30 min in 1 ml of lysis buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 100 μM Na_3VO_4, 2 mM EDTA and 1% Nonidet P-40) containing 10% glycerol, leupeptin (5 μg/ml) and 1 mM phenylmethyl-sulfonyl fluoride. These cell lysates were then centrifuged at 15,000 rpm for 15 min at 4˚C, and the supernatant was harvested after centrifugation. For immunoprecipitation, 20 μl of anti-phosphotyrosine antibody agarose was incubated with supernatant containing 500 μg of protein for 1 h at 4˚C. Immunoprecipitates were washed three times with PBS containing 1% Nonidet P-40 and 100 μM Na_3VO_4, three times with 100 mM Tris HCl, pH 7.5, containing 500 mM $LiCl_2$ and 100 μM Na_3VO_4, and finally twice with 25 mM Tris-HCl, pH 7.5 containing 100 mM NaCl, 1 mM EDTA and 100 μM Na_3VO_4. To

perform the PI3-kinase assay, the immunoprecipitates were resuspended in 100 μl of kinase assay buffer containing 20 mM Tris-HCl, pH 7.6, 75 mM NaCl, 10 mM $MgCl_2$, and 200 μg/ml phosphatidylinositol sonicated in 10 mM Tris-HCl, pH 7.6, 1 mM EGTA, 10 μM ATP and 10 μCi [γ-^{32}P] ATP, and incubated for 20 min at room temperature with constant shaking. The reaction was stopped by the addi-tion of 100 μl of 1 N HCl and 200 μl of $CHCl_3$-methanol (1:1). The samples were centrifuged, and the lower organic phase was harvested and applied to a silica gel TLC plate (Merck) coated with 1% potassium oxalate. TLC plates were developed in $CHCl_3$-CH_3OH-H_2O-NH_4OH (60:47:11.3:2), dried and visualized by autoradiography.

Overexpression of GST-p85 fusion protein and GST pull-down assay

The GST-p85 fusion protein was overexpressed in *E. coli* transformed with the plasmid of the p85α subunit and subcloned into pGEX-2T by induction with 0.1 mM IPTG (isopropyl 1-thio-β,D-galactopyranoside) for 4 h at 37˚C. The overexpressed proteins were purified using glutathione-Sepharose resins as follows. The induced *E. coli* were lysed with PBS buffer containing 1% NP 40 and then these bacterial cell lysates were centrifuged at 15,000 rpm for 15 min at 4˚C. The supernatant was harvested, incubated with glutathione-Sepharose resins for 1 h, and then finally washed with PBS. The expression of the GST-p85 fusion protein was confirmed using SDS-PAGE and immunoblotting after elution by glutathione. The cleavage of the p85 subunit from this fusion protein was done by incubating the eluate with bovine thrombin in a thrombin cleavage buffer for 2 h at 4˚C. The thrombin cleavage buffer contained 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2.5 mM $CaCl_2$, and 0.1% β-mercaptoethanol. For the GST pull-down assay, before binding with GST-p85, glutathione-Sepharose resin was mixed with Sepharose (1:5) to dilute the glutathione-Sepharose resin because of its high capacity for GST-p85 (>1 μg/μl). twenty μl of glutathione-Sepharose/Sepharose resin was incubated with control and insulin-treated 3T3 L1 cell lysates for 5 h at 4˚C. PI3-kinase activity on the resin was measured as described above.

Immunoblotting

The PI3-kinase complexes pulled down by the anti-phosphotyrosine antibody and GST-p85 fusion proteins were dissociated by heating in 50 μl of SDS sample buffer for 10 min at 65˚C. After removal of resins by brief centrifugation, these dissociated samples were heated for 3 min at 100˚C. The SDS sample buffer consisted of 100 mM Tris-HCl, pH 6.8, 4% SDS, 20 mM DTT, 1 mM EDTA, 10% glycerol, 1 mM PMSF, 1 mM sodium orthovanadate and 10 mM phenyl phosphate. Samples were then separated by 8% SDS-PAGE, transferred and immunoblotted with specific antibodies conjugated with horse radish peroxidase. The immunoadsorbed proteins were finally detected with a chemiluminiscence kit.

Results and Discussion

Phosphorylation of the p85 subunit is not a determining factor for PI3-kinase activity in 3T3 L1 fibroblasts

Insulin stimulated PI3-kinase activity seven-fold in 3T3 L1 fibroblasts (Figure 1). Although it has been generally believed that insulin stimulates PI3-kinase activity mainly by recruiting more p85-p110 subunit complexes to the insulin receptor substrate-1 (IRS-1), the detailed molecular mechanism by which PI3-kinase activity is stimulated after protein interaction remains elusive. Recently, it was reported that insulin phosphorylates the p85 subunit at tyrosine residues in CHO-HIR cells (Hayashi

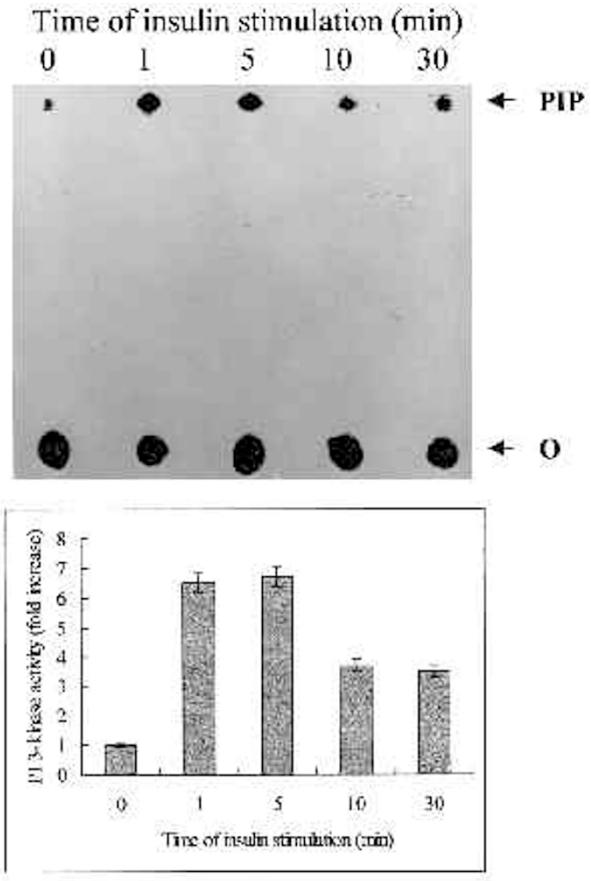

Figure 1. Time course of PI3-kinase activation by insulin in 3T3 L1 fibroblasts. 3T3 L1 fibroblasts were stimulated with 1 μM insulin for indicated period of times, and then PI3-kinases were immunoprecipitated by anti-phosphotyrosine antibody agarose. The PI3-kinase activity was measured as described in 'Materials and Methods'. PIP: phosphatidylinositol-3-monophosphate, O: origin.

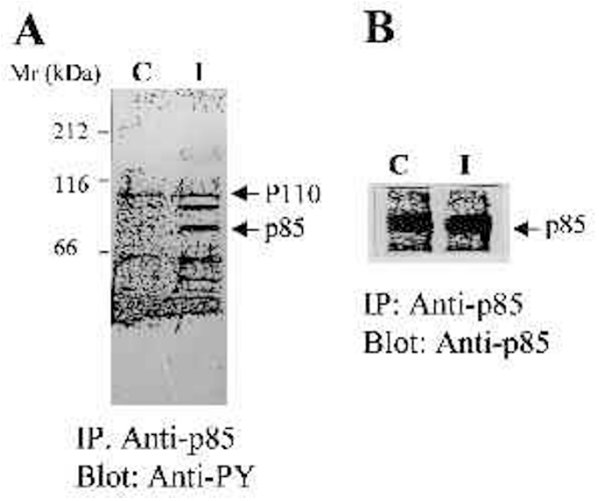

Figure 2. Patterns of tyrosine-phosphorylated proteins in the PI3-kinase complexes immunoprecipitated by anti-p85 antibody. The immunoprecipitates from control and insulin-stimulated 3T3 L1 fibroblasts were electrophoresed on 8% SDS-polyacrylamide gels, transferred and then blotted using anti-phosphotyrosine (PY) antibody (A) and anti-p85 antibody (B). The p110 and p85 subunits were indicated by upper and lower arrows, respectively. C: control cell lysates, I: Insulin stimulated cell lysates.

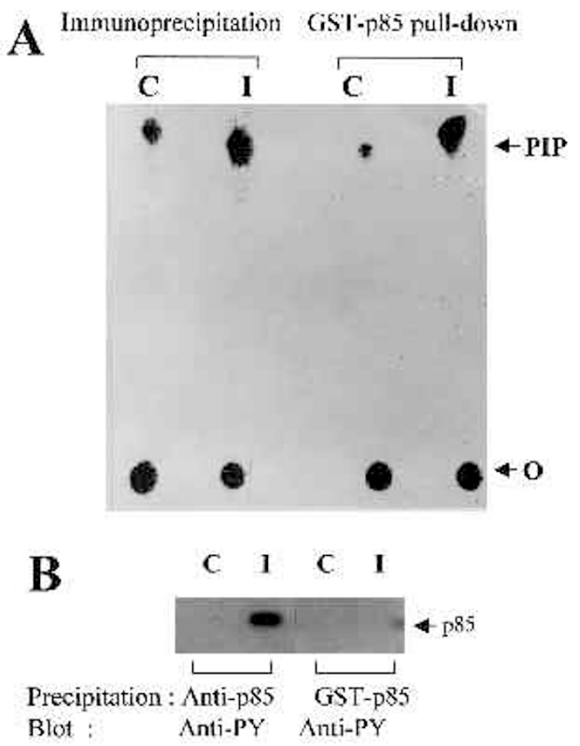

Figure 3. Comparison of PI3-kinase activity and tyrosine phosphorylation of p85 measured by immunoprecipitation and GST pull-down. **(A)** PI3-kinase complexes were harvested from control (C) and insulin stimulated cells (I) using anti-phosphotyrosine antibody and GST-p85 fusion protein immobilized to glutathione-Sepharose resins. The PI3-kinase activity was measured as described in 'Materials and Methods'. PIP: phosphatidylinositol-3-monophosphate, O: origin. **(B)** p85 protein was obtained by either immunoprecipitation or thrombin cleavage of GST-p85. Before thrombin cleavage of GST, GST-p85 fusion protein was incubated with cell lysates for 5 h. The p85 protein was electrophoresed on 8% SDS-PAGE, transferred and then blotted with anti-phosphotyrosine (PY) antibody.

et al., 1993), which, in turn, stimulates its activity (Woscholski *et al*., 1994). Therefore, we attempted to determine whether phosphorylation or other post-translational modifications of p85 subunit affect PI3-kinase activity. To perform this work, we first immunoprecipitated PI3-kinase complexes from both control and insulin-treated cells using an anti-p85 antibody, and then immunoblotted these immuno-precipitates with an anti-phosphotyrosine antibody. The results clearly showed that insulin increases phosphoryl-ation of the p85 subunit at tyrosine residues (Figure 2A), even though the amount of the p85 subunit did not change in control and insulin-stimulated cell lysates (Figure 2B). To clarify the activation mechanism of PI3-kinase, we examined whether tyrosine phosphorylation of the p85 subunit contributes to PI3-kinase activation. Since recombinant protein overexpressed in *E. coli* is not modified by phosphorylation, we assumed that if PI3-kinase activity is maintained even in PI3-kinase complexes pulled down by the GST-p85 fusion protein, post-trans-lational modifications are not functionally significant. It was previously reported that PI3-kinase activity could be obtained after pulling down by this fusion protein (Kotani *et al*, 1994). However, it was not demonstrated how much PI3-kinase activity was harvested by the fusion protein, compared with PI3-kinase activity in immunopre-cipitates. This idea led us to pull down the p110 subunit with both the anti-phosphotyrosine antibody and the GST-p85 fusion protein overexpressed in *E. coli*, and then to compare the PI3-kinase activities harvested by the two methods. After overexpressing the p85 subunit in *E. coli*, we tested whether this protein pulls down PI3-kinase activity. For this purpose, we pulled down PI3-kinase by incubating both control and insulin-stimulated cell lysates with an anti-phosphotyrosine antibody and the GST-p85 fusion protein, immobilized on glutathione-Sepharose resins, and then measured PI3-kinase activity (Figure 3A). The result showed that activation of PI3-kinase was also observed with the GST-pull down assay indicating that PI3-kinase activity pulled down by GST-p85 fusion protein is almost the same as the immunopre-cipitated one. Immunoblotting data show that the concen-tration of the p110 catalytic subunit was also the same, indicating that both immunoprecipitation and GST-p85 pull down precipitate the same amount of p110 (Figure 4A). This result suggests that the p85 subunit in p85-p110 complexes which are formed

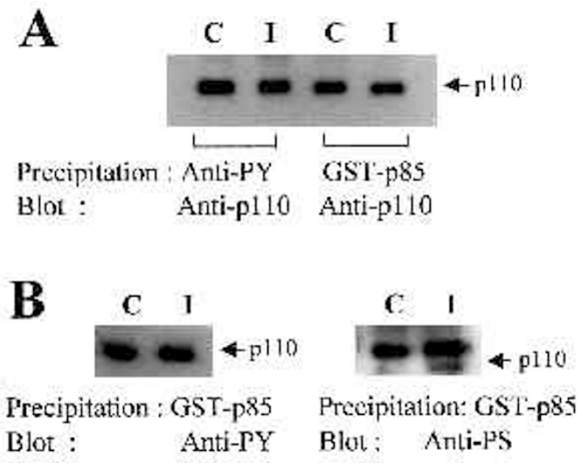

Figure 4. Insulin effects on the heterodimerization of p85-p110 subunits and the phosphorylation of p110 subunits. The amount of heterodimerized p85-p110 subunits was determined by immunoblot of p110 subunits in PI3-kinase complexes prepared with anti-phosphotyrosine antibody and GST-p85 fusion protein **(A)**. To determine the phosphorylation state of p110 subunits, PI3-kinase complexes prepared with GST-p85 fusion protein were immunoblotted with anti-phosphotyrosine (PY) and anti-phosphoserine (PS) antibodies **(B)**. C : control cell lysates, I: Insulin stimulated cell lysates.

intracellularly can be replaced by a GST-p85 fusion protein without significantly changing PI3-kinase activity. The GST-p85 protein was not phosphorylated during incubation with control and insulin-stimulated cell lysates (Figure 3B). Alltogether, these results suggest that phosphorylation of the p85 subunit does not significantly affect PI3-kinase activity.

Analysis of the p110 subunit associated with the GST-p85 fusion protein

Since tyrosine phosphorylation of the p85 subunit did not affect PI3-kinase activity significantly, we next investigated the p110 catalytic subunit of PI3-kinase. Firstly, we attempted to determine whether the p110 subunit exists as either a free monomeric or heterodimeric p110-p85 form in cytosol, and also whether insulin recruits the monomeric p110 subunit to the p85 subunit. To perform this work, we compared amounts of the p110 subunit precipitated by anti-phosphotyrosine antibody and GST pull-down from control and insulin-stimulated cell lysates by immunoblot with the anti-p110 antibody. The immunoprecipitation with anti-phosphotyrosine antibody precipitates only the heterodimeric p110 subunit complexed with p85, whereas GST-p85 fusion protein pulls down both free monomeric and heterodimeric forms of the p110 subunit. Therefore, if monomeric p110 subunit exists in cytosol and insulin recruits the free monomeric p110 subunit to the p85 subunit, immunoprecipitation with an anti-phosphotyrosine antibody would show an increased amount of p110 subunits in insulin-treated cells than in the control, whereas the amount of the p110 subunit pulled down by the GST-p85 fusion protein would be the same in both cell lysates. However, both immunoprecipitation and GST pull-down showed no difference in the amount of p110 subunits in both cells (Figure 4A). Furthermore, the p110 subunits were not left in the supernatant after precipitation by both methods (data not shown). This result indicates that most p110 subunits exist as heterodimeric forms in cytosol. Also, it indicates that insulin activates PI3-kinase activity by recruiting these heterodimeric p85-p110 complexes which are already formed

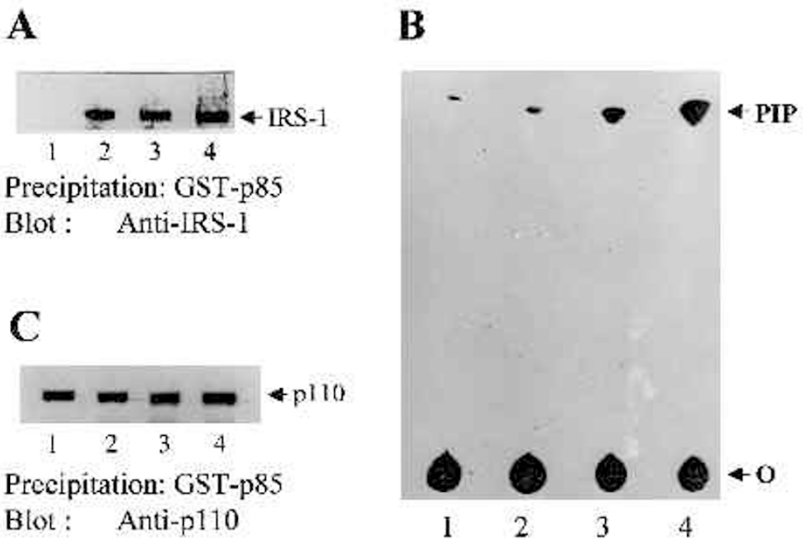

Figure 5. Comparison of time course of *in vitro* association of IRS-1 with GST-p85 fusion protein and PI3-kinase activation. The GST-p85 fusion proteins immobilized to resins were incubated with 3T3 L1 cell lysates without (lane 1) or with insulin stimulation for 20 min, 2 h and 5 h (lane 2-4). After washing, PI3-kinase complexes pulled down by these fusion proteins, PI3-kinase complexes were divided into three groups: The first group was immunoblotted with anti- IRS-1 antibody **(A)** with the arrow indicating IRS-1. The second was used for determination of PI3-kinase activity **(B)**. The third was immunoblotted with anti-p110 antibody **(C)**.

from cytosol to the membrane instead of recruiting more free p110 subunits to p85 subunits. Since it has been believed that the p110 subunit exists in a free monomeric or heterodimeric form with the p85 subunit, this result was rather surprising. Next, we examined whether insulin phosphorylates the p110 subunit on tyrosine and serine residues. For this purpose, we collected the p110 subunits by GST pull-down from control and insulin-treated cell lysates and then immunoblotted with anti-phosphotyrosine and anti-phosphoserine antibodies. The results show that the p110 subunit is phosphorylated on tyrosine and serine residues even in control cells and that the phosphorylation state in insulin-stimulated cells is not different from that in control cells (Figure 4B). The same results were also observed in the immunoprecipitates. Therefore, we concluded that more recruiting of the p110 subunit to the p85 subunit or phosphorylation of this subunit was not involved in the activation of PI3-kinase by insulin.

The significance of IRS-1 in the activation of PI3-kinase

It is well known that insulin recruits IRS-1 to IGF-1 or insulin receptors and then PI3-kinase to IRS-1 through p85, thereby activating PI3-kinase. However, it remains to be determined whether PI3-kinase binding to IRS-1 is sufficient to fully activate PI3-kinase. Since immunoprecipitation with antibodies against each of the p85, p110 subunits or the IRS-1 coimmunoprecipitates all these molecules together, immunoprecipitation with any of these antibodies does not elucidate the functional significance of IRS-1 in the activation of PI3-kinase. Therefore, we decided to replace cellular p85 subunits with GST-p85 fusion proteins immobilized on glutathione-Sepharose resins *in vitro* and then compare PI3-kinase activity with the amount of IRS-1 coprecipitated by the fusion protein after incubation for 20 min, 2 and 5 h. Surprisingly, the results showed that the amount of IRS-1 detected by immunoblot with anti-IRS-1 antibody did not increase significantly with longer incubations (Figure 5A), whereas PI3-kinase activity dramatically increased with longer incubations (Figure 5B). The amount of p110 subunits bound to GST-p85 also did not increase (Figure 5C). These results seem to indicate that heterodimeric PI3-kinase rapidly binds to IRS-1, but this binding is not enough to fully activate PI3-kinase. Therefore, we suggest some other mechanisms are involved in the activation of PI3-kinase even though we could not clarify these. The p85 subunit contains several functional domains, including two SH2 domains, one SH3 domain, a BCR (breakpoint cluster region)-like GTPase activating protein domain, and two different proline-rich regions which represent potential SH3 domain binding sites (Kepeller *et al*., 1994). These domains may have unique functions in the acti-vation of PI3-kinase by binding many cellular proteins. The p110 subunit has functional domains for lipid kinase and protein kinase activity, suggesting that this subunit may bind unknown proteins for full activation of PI3-kinase activity. This study of GST-p85 pull down *in vitro* suggests that the role of this binding protein is not membrane targeting of PI3-kinase but directly affects PI3-kinase activation.

Another important aspect of our study is that this GST-p85 fusion protein can be successfully used for PI3-kinase assay instead of immunoprecipitation with anti-phosphotyrosine or PI3-kinase antibodies. Immunoprecipitation with anti-phosphotyrosine or anti-PI3-kinase antibodies has been widely used for PI3-kinase assay. One of the disadvantages of this immunoprecipitation is that antibodies used for immunoprecipitation are expensive. Recently, a novel, rapid and sensitive method for the assay of the PI3-kinase product, phosphatidylinositol 3,4,5-triphosphate (PI-3,4,5-P_3), was developed to overcome the disadvantages of PI3-kinase assay by a high dose of radioisotopes (Van der Kaay *et al*., 1997). However, this new isotope dilution assay can not be employed for *in vitro* PI3-kinase assay because the method does not apply to PI3-kinase products of PI-3-P and PI-3,4-P2. In this study, We demonstrated that the GST-p85 fusion protein may be a good alternative to antibodies for routine PI3-kinase assay.

In summary, we showed that phosphorylation of p85 on tyrosine residues and p110 subunits on serine and tyrosine residues are not involved in the activation of PI3-kinase. It was also shown that insulin does not activate PI3-kinase through more recruitment of the p110 subunit to the p85 subunit. However, we showed that insulin increases PI3-kinase activity probably by recruiting other binding protein(s) to heterodimeric p85 and p110 subunits as well as recruiting these subunits to IRS-1.

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