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Five isoforms of the phosphatidylinositol 3-kinase regulatory subunit exhibit different associations with receptor tyrosine kinases and their tyrosine phosphorylations

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Abstract There are five isoforms of the regulatory subunit for the heterodimeric type of phosphatidylinositol 3-kinase. These five regulatory subunit isoforms were overexpressed using an adenovirus transfection system, and their own tyrosine phosphorylations and associations with various tyrosine kinase receptors were investigated. When overexpressed in CHO-PDGFR cells, the associations of these regulatory subunit isoforms with the platelet-derived growth factor receptor were similar. However, when overexpressed in CHO-IR cells, p55γ exhibited a significantly lower ability to bind with IRS-1 upon insulin stimulation, as compared with other regulatory subunit isoforms. Furthermore, p55α and p55γ were found to be tyrosine-phosphorylated. Finally, interestingly, when overexpressed in CHO-EGFR cells or A431 cells and stimulated with epidermal growth factor (EGF), phosphorylated EGF receptor was detected in p85α, p85β and p50α immunoprecipitates, but not in p55α and p55γ immunoprecipitates. In addition, EGF-induced tyrosine phosphorylation was observed in p85α, p85β, p55α and p55γ, but not in p50α, immunoprecipitates. Thus, each regulatory subunit exhibits specific responses regarding both the association with tyrosine-phosphorylated substrates and its own tyrosine phosphorylation. These results suggest that each isoform possesses specific roles in signal transduction, based on its individual tyrosine kinase receptor.

Key words: Phosphatidylinositol 3-kinase; Regulatory subunit; Platelet-derived growth factor; Epidermal growth factor; Insulin

1. Introduction

Many receptor tyrosine kinases (RTKs) transduce their signals via specific interactions with SH2 domain-containing proteins [1]. The SH2 domain-containing regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase) has been shown to bind directly to the tyrosine-phosphorylated YXXM motif of many activated RTKs [2,3]. To date, five mammalian regulatory subunit isoforms have been identified, including two 85 kDa proteins (p85α and p85β), two 55 kDa proteins (p55α and p55γ), and one 50 kDa protein (p50α). All of these isoforms share two SH2 domains and an inter-SH2 domain but contain different NH_2-terminal sequences. The most well-known 85 kDa isoforms, p85α and p85β, contain SH3 and *bcr* homology domains in their N-termini [4–7]. The recently cloned 55 kDa isoforms, p55α and p55γ, contain a unique 34 amino acid sequence in their N-termini [8–10]. The 50 kDa isoform, an alternative splice variant of the p85α gene, contains only a unique 6 amino acid sequence in its N-terminal portion, which is apparently too short to interact with other molecules [11,12].

In this study, we overexpressed these regulatory subunit isoforms in A431 cells and Chinese hamster ovary (CHO) cells overexpressing three distinct RTK types, insulin receptor (IR), platelet-derived growth factor receptor (PDGFR) and epidermal growth factor receptor (EGFR), and examined the associations of the regulatory subunits with these three RTKs in response to the stimulation of each ligand. In addition, the tyrosine phosphorylations of these regulatory subunit isoforms were also investigated. We herein show these differences in responses among the PI 3-kinase regulatory subunit isoforms for the first time. The results suggest that each regulatory subunit isoform plays a role in the response to individual stimuli.

2. Materials and methods

2.1. Cell culture and preparation of cell lysates

CHO (K1) cells stably overexpressing human IR (CHO-IR) were prepared as described previously [11]. CHO cells overexpressing PDGF-β receptor (CHO-PDGFR) or EGFR (CHO-EGFR) were generously provided by Prof. Y. Ebina (Institute for Enzyme Research, University of Tokushima, Japan). These CHO cells were maintained in Ham's F-12 containing 10% fetal calf serum at 37°C in 5% CO_2. A431 cells, a cell line derived from a human carcinoma cell line, were grown in Dulbecco's modified Eagle medium containing 10% fetal calf serum at 37°C in 5% CO_2. Prior to exposure to growth factors, the medium was replaced with serum-free medium containing 0.2% bovine serum albumin and the cells were incubated for 18 h. Then, the corresponding growth factors were added to the medium (50 μg/ml for PDGF, 100 nM for insulin and 100 μg/ml for EGF) and the cells were incubated for 10 min at 37°C. After stimulation, the medium was removed and the cells were washed twice with ice-cold phosphate-buffered saline, and lysed in 5 ml of ice-cold lysis buffer containing 1% Nonidet P-40, 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 100

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μM sodium orthovanadate, and 1 mM phenylmethylsulfonyl fluoride. The lysate was cleared by centrifugation at 14 000×*g* for 10 min. The resulting supernatants were used for the experiments.

2.2. Transient expression of regulatory subunits of PI 3-kinase with an adenovirus expression system

The cassette cosmid for constructing recombinant adenovirus, pAdex1wt, was a generous gift from Dr. Izumi Saito (Institute of Medical Science, University of Tokyo). The cDNAs encoding the full-length amino acid sequences of p85α, p85β, p55α, p55γ and p50α, as well as the HA-tag amino acid sequence (YPYDVPDYA) at each C-terminus, were ligated into the *Swa*I sites of pAdex1wt. The cDNA encoding p110α as well as the GLUT2-tag amino acid sequences corresponding to residues 510–524 of human GLUT2 were also ligated similarly. Recombinant adenoviruses were obtained as previously described [13]. A431 cells and CHO cells overexpressing each RTK corresponding to one 100 mm plate (10^7 cells) were infected with these viruses for 1 h, then grown for 48 h. The amounts of each regulatory protein expressed were evaluated by immunoblotting, using anti-HA antibody (12CA5), and the amounts were confirmed to be nearly.

2.3. Immunoprecipitation and Western blotting

The resulting cell lysates were incubated with appropriate antibodies. αEGFR, αPDGFR and αIR were obtained from Sigma RBI. αHA (12CA5) was obtained from Boehringer Mannheim. Phospho-specific AKT1 (Ser473) antibody was obtained from New England Biolabs. αGLUT2-tag and αIRS-1 were as previously described [14,15]. Protein G-Sepharose beads were used to precipitate the immune complexes. The beads were washed three times with lysis buffer and the immunoprecipitated proteins were separated from the beads by boiling in Laemmli buffer. The beads were removed by centrifugation, and supernatants were subjected to SDS–PAGE. Immunoblotting, to detect phosphotyrosine proteins, was performed using anti-phosphotyrosine antibody (4G10) as previously described [16]. These experiments were performed three times.

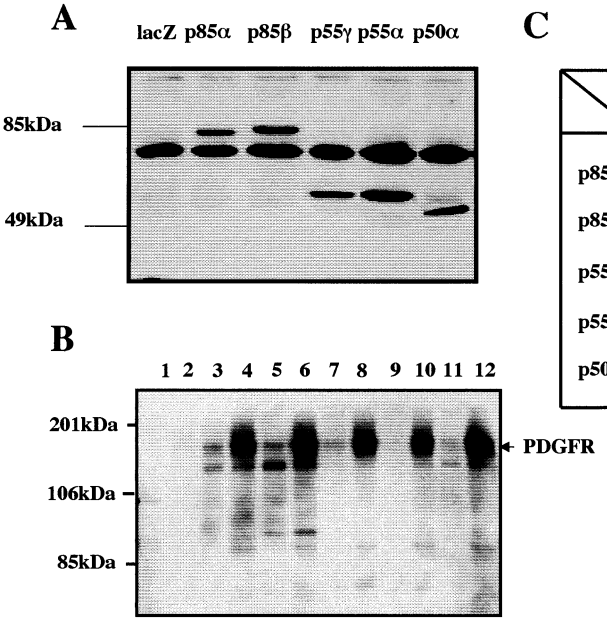
2.4. PI 3-kinase assay

The resulting cell lysates were incubated with anti-HA antibody. The protein G-Sepharose beads were used to precipitate the immune complexes. The presence of PI 3-kinase activity in immune complexes was determined as described previously [17]. Radioactivity was measured using a Molecular Imager instrument.

3. Results and discussion

3.1. Five regulatory subunits associate with PDGFR similarly

Several studies have shown a direct association between tyrosine-phosphorylated PDGFR and p85 regulatory subunits of PI 3-kinase [18,19]. In this study, each regulatory subunit isoform was overexpressed in CHO-PDGFR cells. The amounts of the regulatory protein expressed in CHO cells were essentially equal (Fig. 1A). We stimulated these cells with PDGF, and found PDGFR with a molecular weight of 180 kDa in the immunoprecipitate of each regulatory subunit (Fig. 1B). PDGFRs associated with each regulatory protein were observed to be phosphorylated when blotted with anti-phosphotyrosine antibody (data not shown). We calculated the ratios of the PDGFR to each regulatory subunit protein as shown in Fig. 1C. Upon PDGF stimulation, there was no apparent difference in either the association of the five regulatory subunits with PDGFR, or tyrosine phosphorylation of the regulatory subunits. These associations were also demonstrated by reversing the immunoprecipitation experiments, in which comparable amounts of each regulatory subunit were detected in PDGFR immunoprecipitates (data not shown). These results suggest that all of these regulatory subunits sim-



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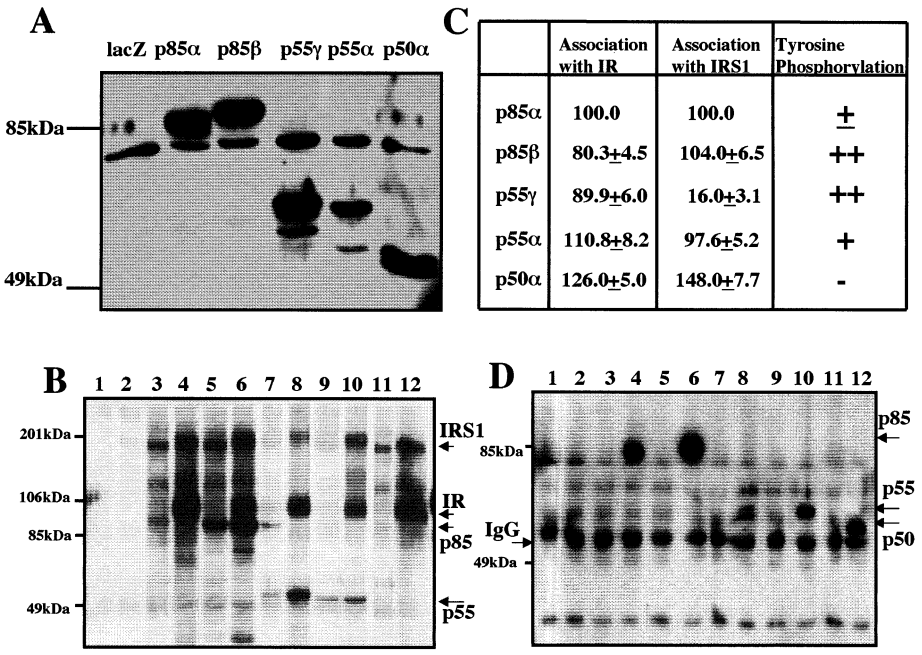
	Association with PDGFR	Tyrosine Phosphorylation
p85α	100.0	-
p85β	98.1±7.2	-
p55γ	92.3±9.9	-
p55α	87.5±7.4	-
p50α	109±7.1	-

Fig. 1. (A) Immunoblotting of the expressed regulatory subunit proteins using anti-HA antibody. The cell lysates from CHO-PDGFR cells, transiently expressing each of the regulatory subunits of PI 3-kinase (lane 1: Lac Z, lane 2: p85α, lane 3: p85β, lane 4: p55γ, lane 5: p55α, lane 6: p50α), were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads. The beads were washed and the proteins were separated from the beads by boiling in Laemmli buffer. The supernatants were then subjected to SDS–PAGE. To detect expressed regulatory proteins, immunoblotting was performed using anti-HA antibody. (B) PDGFR in immunoprecipitates of each regulatory subunit of PI 3-kinase. CHO-PDGFR, transiently overexpressing each regulatory subunit of PI 3-kinase (lanes 1, 2: Lac Z, lanes 3, 4: p85α, lanes 5, 6: p85β, lanes 7, 8: p55γ, lanes 9, 10: p55α, lanes 11, 12: p50α), were incubated for 10 min in the presence (lanes 2, 4, 6, 8, 10, 12) or in the absence (lanes 1, 3, 5, 7, 9, 11) of 50 μg/ml PDGF. The resulting cell lysates were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads, and subjected to SDS–PAGE. Immunoblotting was performed using anti-PDGFR antibody. (C) The ratios of PDGFR to each regulatory subunit protein, measured densitometrically, were calculated. Values are shown as percentages of the ratio of the phosphorylated proteins to p85α regulatory subunits. Data shown are means ± S.E.M.



	Association with IR	Association with IRS1	Tyrosine Phosphorylation
p85α	100.0	100.0	±
p85β	80.3±4.5	104.0±6.5	++
p55γ	89.9±6.0	16.0±3.1	++
p55α	110.8±8.2	97.6±5.2	+
p50α	126.0±5.0	148.0±7.7	-

Fig. 2. (A) Immunoblotting of the expressed regulatory subunits using anti-HA antibody. The cell lysates from CHO-IR cells, transiently expressing each of the regulatory subunits of PI 3-kinase (lane 1: Lac Z, lane 2: p85α, lane 3: p85β, lane 4: p55γ, lane 5: p55α, lane 6: p50α), were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads, and then subjected to SDS–PAGE. Immunoblotting was performed using anti-HA antibody. (B) The phosphotyrosine proteins in immunoprecipitates of each regulatory subunit of PI 3-kinase. CHO-IR cells, transiently overexpressing each regulatory subunit of PI 3-kinase (lanes 1, 2: Lac Z, lanes 3, 4: p85α, lanes 5, 6: p85β, lanes 7, 8: p55γ, lanes 9, 10: p55α, lanes 11, 12: p50α), were incubated for 10 min in the presence (lanes 2, 4, 6, 8, 10, 12) or in the absence (lanes 1, 3, 5, 7, 9, 11) of 100 nM insulin. The resulting cell lysates were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads, and subjected to SDS–PAGE. Immunoblotting, to detect phosphotyrosine proteins associated with each of the expressed regulatory subunits, was performed using anti-phosphotyrosine antibody (4G10). (C) The ratios of the phosphorylated proteins to each regulatory subunit protein, measured densitometrically, were calculated. Values are shown as percentages of the ratio of the phosphorylated proteins to p85α regulatory subunits. Data shown are means ± S.E.M. (D) Each PI 3-kinase regulatory subunit in IRS-1 immunoprecipitates. The resulting cell lysates were immunoprecipitated with anti-IRS-1 antibody, and subjected to SDS–PAGE. Immunoblotting was performed using anti-HA antibody.

ilarly transmit the signal downstream via association with PDGFR.

3.2. Lower affinity of p55γ regulatory subunit for IRS-1 than for other subunits

Tyrosine-phosphorylated proteins associating with overexpressed regulatory subunits of PI 3-kinase in CHO-IR cells upon insulin stimulation were investigated by the same method. The amounts of regulatory protein expressed in CHO cells were essentially equal (Fig. 2A). As shown in Fig. 2B, there were two major phosphorylated proteins with molecular weights of 180 kDa and 95 kDa, corresponding to the tyrosine-phosphorylated IRS-1 and IR. IRS-1 is one of the major IR kinase substrates that undergoes tyrosine phosphorylation and reportedly possesses four putative tyrosine residues which bind to the SH2 domain in the regulatory subunits of PI 3-kinase [20,21]. On the other hand, the IR also possesses a motif containing Y1322, capable of binding to PI 3-kinase, in its C-terminus [3,22]. Thus, besides PI 3-kinase binding to one of IRS-1 and IR, it is possible that IR/IRS-1/regulatory subunits of PI 3-kinase form stable ternary complexes [23].

As shown in Fig. 2B, the amount of IRS-1 bound to p55γ was much smaller than that bound to the other regulatory subunits, while there were no significant differences in the amount of IR bound to the regulatory subunit among the isoforms. IRS-1/regulatory subunit interactions were also demonstrated by reversing the immunoprecipitation experiments, in which small amounts of p55γ were observed in the IRS-1 immunoprecipitates (Fig. 2D). The lowest affinity of p55γ for IRS-1 was also observed in similar experiments using HepG2 cells, in a previous study [11]. This phenomenon suggests that p55γ may mediate insulin signaling mainly via association with IR rather than IRS-1. In addition, a recent study demonstrated a tight association between insulin-like growth factor receptor and p55γ [24], also suggesting a crucial role for p55γ as an adapter protein for insulin or the IGF receptor, rather than IRS-1 protein.

The tyrosine phosphorylations of p85β, p55α and p55γ were apparent in CHO-IR cells upon stimulation with insulin. Although the tyrosine phosphorylation of p85β was observed irrespective of insulin stimulation, taking the absence of phosphorylation of p85β expressed in control CHO (data not shown) or CHO-PDGF cells into consideration, the tyrosine phosphorylation of p85β is presumably induced by IR even in the absence of insulin stimulation. Regarding the tyrosine phosphorylation of p55α and p55γ, it has been shown that Y29 and Y2/29 in the unique N-terminal sequence were the tyrosine residues phosphorylated by IR and Tec tyrosine kinase, respectively [25]. Previous studies found the putative phosphorylation sites to be Tyr-368, 580 and 607 in p85α [26]. As these tyrosine-containing motifs are recognized by various SH2 proteins, it is possible that these phosphorylation sites are linked to other SH2-containing molecules or mediate the dimerization of regulatory subunits. As yet, no informa-

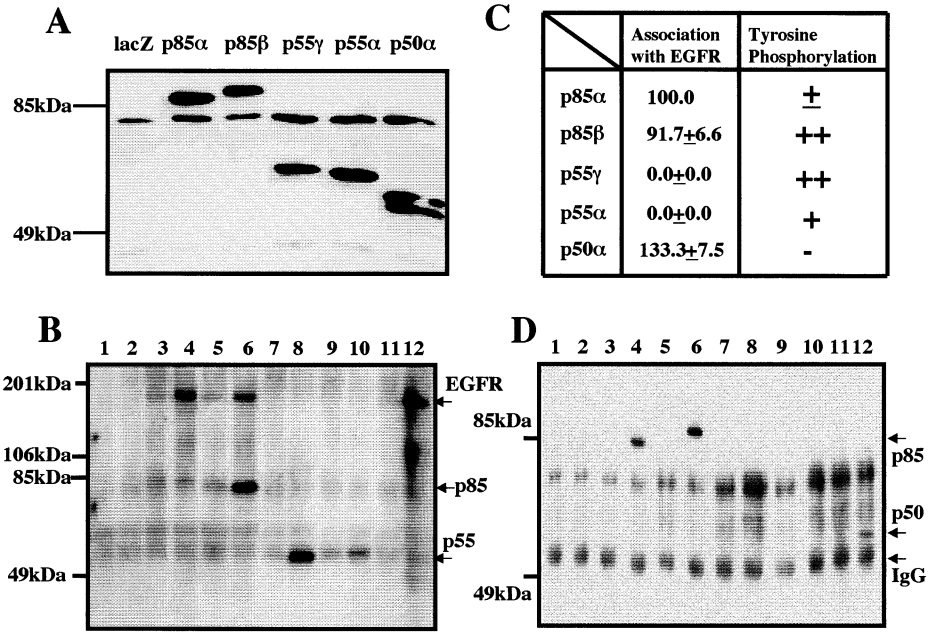


Fig. 3. (A) Immunoblotting of the expressed regulatory subunits using anti-HA antibody. The cell lysates from CHO-EGFR cells, transiently expressing each of the regulatory subunits of PI 3-kinase (lane 1: Lac Z, lane 2: p85α, lane 3: p85β, lane 4: p55γ, lane 5: p55α, lane 6: p50α), were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads, and then subjected to SDS–PAGE. Immunoblotting was performed using anti-HA antibody. (B) The phosphotyrosine proteins in immunoprecipitates of each regulatory subunit of PI 3-kinase. CHO-EGFR cells, transiently overexpressing each regulatory subunit of PI 3-kinase (lanes 1, 2: Lac Z, lanes 3, 4: p85α, lanes 5, 6: p85β, lanes 7, 8: p55γ, lanes 9, 10: p55α, lanes 11, 12: p50α), were incubated for 10 min in the presence (lanes 2, 4, 6, 8, 10, 12) or in the absence (lanes 1, 3, 5, 7, 9, 11) of 100 μg/ml for EGF. The resulting cell lysates were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads, and subjected to SDS–PAGE. Immunoblotting, to detect phosphotyrosine proteins associated with each of the expressed regulatory subunits, was performed using anti-phosphotyrosine antibody. (C) The ratios of the phosphorylated EGFR to each regulatory subunit protein, measured densitometrically, were calculated. Values are shown as percentages of the ratio of the phosphorylated proteins to p85α regulatory subunits. Data shown are means ± S.E.M. (D) Each PI 3-kinase regulatory subunit in EGFR immunoprecipitates. The resulting cell lysates were immunoprecipitated with anti-EGFR antibody, and subjected to SDS–PAGE. Immunoblotting was performed using anti-HA antibody.

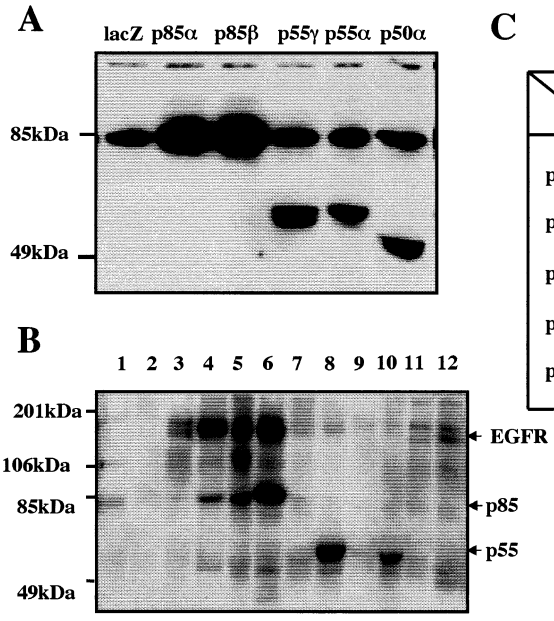
tion is available on the tyrosine residue phosphorylated in the regulatory subunit of PI 3-kinase. Further study is thus necessary to clarify the biological significance of these tyrosine phosphorylations.

3.3. EGFR associates with p85 and p50 regulatory subunits, but not p55, upon EGF stimulation

Tyrosine-phosphorylated proteins associating with overexpressed regulatory subunits in CHO-EGFR cells upon EGF stimulation were similarly assessed by anti-phosphotyrosine immunoblotting. The amounts of the regulatory protein expressed in CHO cells were essentially equal (Fig. 3A). Upon EGF stimulation, one major phosphorylated protein with a molecular weight of 180 kDa was observed in immunoprecipitates of p85 and p50 (Fig. 3B). This protein corresponds to the autophosphorylated EGFR. EGFR/regulatory subunit interactions were detected by reversing the immunoprecipitation experiments, in which only p85 and p50 regulatory subunits were detected in the EGFR immunoprecipitates (Fig. 3D). The mechanism of EGF-induced PI 3-kinase activation is somewhat complicated. EGFR lacks a tyrosine-phosphorylated YXXM motif and direct association of PI 3-kinase with EGFR is controversial [27,28]. Some reports suggest that EGFR does bind with p85 regulatory subunits [29–31], although the affinity is much weaker than that with many other tyrosine kinase receptors such as PDGFR. On the other hand, ErbB3, c-Cbl and Gab1, substrates of EGFR, have been shown to associate with and activate PI 3-kinase in response to EGF stimulation [16,32,33]. Which of these adapter proteins is/are involved in PI 3-kinase association would differ among cell types. For example, in A431 cells, EGF promotes the association of PI 3-kinase with EGFR-transactivated ErbB3 [28], while in PC12 or A549 cells, c-Cbl has been suggested to serve as an adapter protein for EGF-induced PI 3-kinase activation [32].

In CHO-EGFR cells, tyrosine phosphorylation of EGFR, but not of Gab1 or c-Cbl (data not shown), was observed in response to EGF stimulation. Though the reason for EGFR not being detected in the p55 regulatory subunit immunoprecipitates was not clear, we speculate that the N-terminal portion of p55, which is the phosphorylation site and also shows a high affinity for microtubules [34], may inhibit continuous association with EGFR. Interestingly, both p55α and p55γ were tyrosine-phosphorylated in response to EGF stimulation (Fig. 3B), suggesting that the p55 regulatory subunits of PI 3-kinase may also be involved in activities downstream from EGF signaling. We speculate that after being tyrosine-phosphorylated by EGFR, p55α and p55γ may be removed from EGFR and recruited to a different portion of the cell.

We next examined whether the heterodimer p85–p110 also binds to EGFR. When p110α was overexpressed together with each regulatory subunit in CHO-EGFR cells, no EGFR was detectable in the p110α immunoprecipitates, even in the presence of EGF (data not shown). These results agree with a previous report [4] showing that free p85, but not the p85–p110 complex, binds to EGFR.



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	Association with EGFR	Tyrosine Phosphorylation
p85α	100.0	±
p85β	98.7±7.5	++
p55γ	3.0±1.1	++
p55α	1.8±0.4	+
p50α	33.5±5.5	-

Fig. 4. (A) Immunoblotting of the expressed regulatory subunits using anti-HA antibody. The cell lysates from A431 cells, transiently expressing each of the regulatory subunits of PI 3-kinase (lane 1: Lac Z, lane 2: p85α, lane 3: p85β, lane 4: p55γ, lane 5: p55α, lane 6: p50α), were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads, and then subjected to SDS–PAGE. Immunoblotting was performed using anti-HA antibody. (B) The phosphotyrosine proteins in immunoprecipitates of each regulatory subunit of PI 3-kinase. A431 cells, transiently overexpressing each regulatory subunit of PI 3-kinase (lanes 1, 2: Lac Z, lanes 3, 4: p85α, lanes 5, 6: p85β, lanes 7, 8: p55γ, lanes 9, 10: p55α, lanes 11, 12: p50α), were incubated for 10 min in the presence (lanes 2, 4, 6, 8, 10, 12) or in the absence (lanes 1, 3, 5, 7, 9, 11) of 100 μg/ml for EGF. The resulting cell lysates were incubated with anti-HA antibody covalently coupled to protein A-Sepharose beads, and subjected to SDS–PAGE. Immunoblotting, to detect phosphotyrosine proteins associated with each of the expressed regulatory subunits, was performed using anti-phosphotyrosine antibody. (C) The ratios of the phosphorylated EGFR to each regulatory subunit protein, measured densitometrically, were calculated. Values are shown as percentages of the ratio of the phosphorylated proteins to p85α regulatory subunits. Data shown are means ± S.E.M.

Similar experiments were performed using A431 cells, which endogenously express members of the EGFR family, including EGFR and ErbB3. The amounts of regulatory subunits expressed in A431 cells were essentially equal (Fig. 4A). The phosphorylated proteins with an approximate molecular weight of 180 kDa are likely to correspond to EGFR Fig. 4B), although the possibility that this band also includes the transactivated ErbB3 cannot be excluded as previously described [27]. Nevertheless, in A431 cells, we obtained very similar results regarding both the association with EGFR and tyrosine phosphorylation of the regulatory subunit. Taken together, these observations strongly support the conclusion that p85α, p85β and p50α associate directly with EGFR, and thereby activate PI 3-kinase, while the motif containing phosphorylated tyrosine in p55α and p55γ may play a role in a cellular compartment distinct from EGFR.

3.4. Downstream signaling via each PI 3-kinase regulatory subunit

We measured the PI 3-kinase activities associated with each regulatory subunit, upon stimulation of each ligand, to investigate whether these activities correlate with co-immunoprecipitation data. We previously reported each isoform-associated PI 3-kinase activation in response to insulin, using adenovirus transient expression into CHO-IR cells [11]. In this study, we performed similar experiments using CHO-PDGFR and CHO-EGFR cells. With PDGF stimulation, the fold increases in PI 3-kinase activation were 2.4, 1.3, 2.0, 3.6 and 10.3 for p85α, p85β, p55γ, p55α and p50α, respectively (Fig. 5A), whereas with EGF stimulation, the fold increases in PI 3-kinase activation were 2.4, 1.6, 0.9, 0.9 and 6.8 for p85α, p85β, p55γ, p55α and p50α, respectively (Fig. 5B). The dissociation between PI 3-kinase activation and the binding data can be explained as follows; although each regulatory subunit isoform has a similar ability to associate with the p110α catalytic subunit, the basal PI 3-kinase activities associated with each regulatory subunit differed significantly. For example, p50α-associated PI 3-kinase activity in the absence of ligand was lower than those of the other isoforms, resulting in an apparent marked increase in p50α-associated PI 3-kinase activation.

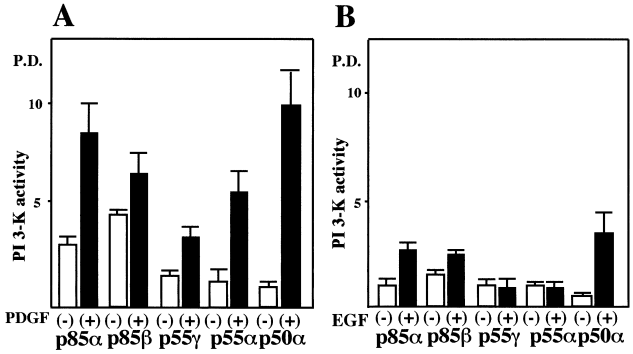

Fig. 5. Ligand-induced activation of PI 3-kinase associated with each regulatory subunit in CHO-PDGFR (A) and CHO-EGFR (B). We overexpressed each regulatory subunit in CHO-PDGFR or CHO-EGFR cells. After each ligand treatment, the resulting cell lysates were immunoprecipitated with anti-HA antibody and assayed for PI 3-kinase activity as previously described. P.D., pixel density.

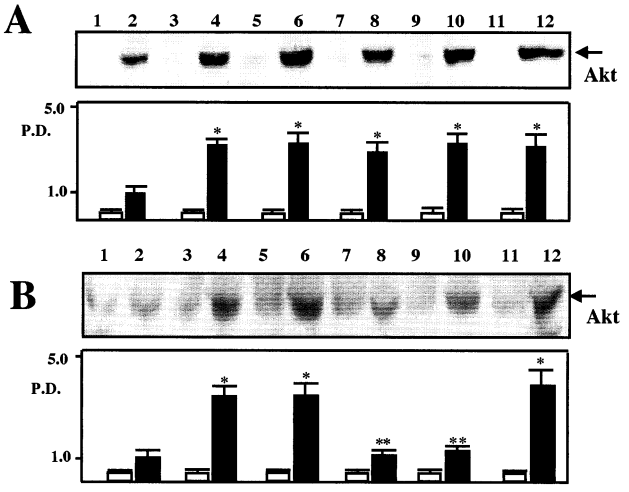

Fig. 6. Immunoblotting for the activated Akt with the stimulation of each ligand. CHO-PDGFR (data not shown), CHO-IR (A) and CHO-EGFR cells (B) overexpressing each regulatory subunit of PI 3-kinase (lanes 1, 2: Lac Z, lanes 3, 4: p85α, lanes 5, 6: p85β, lanes 7, 8: p55γ, lanes 9, 10: p55α, lanes 11, 12: p50α) were treated with each ligand and the resulting cell lysates were subjected to SDS–PAGE. Immunoblotting to detect activated Akt was performed using phospho-specific AKT1 (Ser473) antibody. $*P < 0.05$, all compared with Lac Z-expressing cells with stimulation of each ligand (lane 2). $**P < 0.05$, all compared with EGF-stimulated p85α-expressing cells (B, lane 4). P.D., pixel density.

Secondly, the degree of PI 3-kinase activity enhancement, which is induced by the association with receptors, also differed significantly among the isoforms. For example, p55γ-associated PI 3-kinase activity is not particularly high in comparison with that associated with p85α.

Ligand-induced activation of PI 3-kinase leads to the generation of second messengers, PI(3,4)P2 and PI(3,4,5)P3, which play crucial roles in the activation of PKB/Akt [35]. To investigate whether there is differential downstream activation of PI 3-kinase targets, we performed immunoblotting to visualize the serine phosphorylation of Akt using phospho-specific AKT1 (Ser473) antibody. In CHO-PDGFR (data not shown) and CHO-IR cells (Fig. 6A), the level of Akt phosphorylation was significantly enhanced by the overexpression of each regulatory subunit isoform, and the degree of enhancement did not differ significantly among the five regulatory isoforms. p55γ exhibited lower association with IRS-1, but a similar association with the IR, which may be the reason for p55γ enhancing insulin-induced Akt phosphorylation to an extent similar to that with the other isoforms. In CHO-EGFR cells, the overexpression of p85α, p85β and p50α resulted in an apparent enhancement of EGF-induced Akt phosphorylation, while overexpressing p55α and p55γ did not (Fig. 6B). These results suggest the importance of the associations between regulatory subunits and the RTK, with respect to the phosphorylation of Akt via PI 3-kinase activation.

In conclusion, this is the first report clearly demonstrating that the five isoforms of the PI 3-kinase regulatory subunit exhibit different associations with RTKs, as well as differing in their tyrosine phosphorylations. Although the physiological significance of tyrosine phosphorylation in the regulatory subunit remains unknown, we believe that the different associations with the individual RTKs are important. Thus, we speculate that the combination of an individual RTK and the expression level of each regulatory subunit may determine the degree of PI 3-kinase activation in response to the corresponding growth factor stimulation.

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