Tyrosine 508 of the 85-Kilodalton Subunit of Phosphatidylinositol 3-Kinase Is Phosphorylated by the Platelet-Derived Growth Factor Receptor†

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ABSTRACT: The mechanisms by which growth factors and oncogenic agents activate phosphatidylinositol 3-kinase (PI3 kinase) are unknown. Previously, we reported that the 85-kDa regulatory subunit of PI3 kinase is tyrosine-phosphorylated both *in vitro* by the platelet-derived growth factor β-receptor (PDGFR) tyrosine kinase and in fibroblasts in response to PDGF. As a first step in determining the role of tyrosine phosphorylation in PDGF signaling through PI3 kinase, we investigated which tyrosines on p85 are phosphorylated by the PDGFR. Recombinant p85 was phosphorylated with recombinant PDGF receptors, and tryptic phosphopeptides were purified by HPLC and analyzed by Edman degradation. By this approach and by mutational analysis, Y508 was identified as the major *in vitro* phosphorylation site. Tryptic phosphopeptide mapping demonstrated Y508 to also be phosphorylated *in vivo* in COS cells. Comparison of these data with a previous report [Hayashi, H., Nishioka, Y., Kamohara, S., Kanai, F., Ishii, K., Fukui, Y., Shibasaki, F., Takenawa, T., Kido, H., Katsunuma, N., & Ebina, Y. (1993) *J. Biol. Chem. 268,* 7107–7117] suggests that p85 is phosphorylated differently by the PDGF and insulin receptor tyrosine kinases. Therefore, p85 may be regulated differently by PDGF and insulin. Mapping of phosphorylation sites on p85 may lead to new insights into the regulation of signal transduction through PI3 kinase.

Phosphatidylinositol 3-kinase (PI3 kinase)[1] is an enzyme which participates in the induction of mitogenesis and cell transformation by several growth factors and oncogenic agents. For example, studies of mutants of the PDGF receptor (Escobedo et al., 1988; Coughlin et al., 1989; Fantl et al., 1992) and of polyomavirus middle T antigen, $pp60^{v\text{-}src}$, and $pp130^{gag\text{-}abl}$ demonstrate a strong correlation between PI3 kinase activity and mitogenesis or cell transformation [reviewed in Cantley et al., (1991)]. Although it is unknown precisely how PI3 kinase participates in the control of cell growth, it is thought to generate a novel class of second-messenger phospholipids by phosphorylating phosphatidylinositol at the D-3′ position of the inositol ring (Whitman et al., 1988).

PI3 kinase is a heterodimer consisting of an 85-kDa subunit (p85) and a 110-kDa subunit (p110) (Carpenter et al., 1989; Morgan et al., 1990; Shibasaki et al., 1991). It has been proposed that p110 is the catalytic subunit, because it contains PI3 kinase activity when expressed in insect or mammalian cell systems (Shibasaki et al., 1991; Hiles et al., 1992). p85 has been considered to be an adapter or "regulatory" subunit, because it mediates the interaction of p110 with activated growth factor receptors and oncoproteins but does not have PI3 kinase activity itself (Kaplan et al., 1987; Escobedo et al., 1991a,b; Otsu et al., 1991). The p85 molecule consists of several functional domains, including an SH3 (for *src* homology) domain, two SH2 domains, a domain with homology to the breakpoint cluster region (bcr), and a region between the two SH2 domains known as the inter-SH2 region (Escobedo et al., 1991b; Skolnik et al., 1991). The SH2 domains mediate interaction of p85 with tyrosine-phosphorylated targets such as the activated PDGF receptor (Hu et al., 1992; Klippel et al., 1992; McGlade et al., 1992), while the inter-SH2 region is responsible for association of p85 with the catalytic subunit of PI3 kinase, p110 (Klippel et al., 1993). The proteins which interact with the other domains of p85 are presently unknown.

PI3 kinase products increase in cells treated with PDGF or transformed by polyomavirus (Auger et al., 1989; Serunian et al., 1990; Hawkins et al., 1992), suggesting that the association of PI3 kinase with the PDGF receptor or middle T antigen regulates the activity of PI3 kinase (Susa et al., 1992). One of the most important questions concerning the role of PI3 kinase in growth control is how p85 and p110 are regulated by growth factor receptors or deregulated by oncoproteins. We previously reported that p85 is tyrosine-phosphorylated at high stoichiometry in response to PDGF treatment *in vivo* (Kavanaugh et al., 1992). P85 is also tyrosine-phosphorylated in insulin-stimulated cells (Hayashi et al., 1991, 1992, 1993), in cells transformed by polyomavirus (Kaplan et al., 1987; Carpenter et al., 1989), and in cells stimulated by nerve growth factor (Soltoff et al., 1992). Tyrosine phosphorylation of p85 is likely to regulate its activity or its association with other proteins in the signaling complex. To begin to address the role of tyrosine phosphorylation in signal transduction through PI3 kinase, we determined which tyrosines on p85 are phosphorylated by the PDGF receptor. In this report, we demonstrate that tyrosine 508 of p85 is a major phosphorylation site *in vitro* for the PDGF receptor and is phosphorylated *in vivo* in COS cells.

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[1] Abbreviations: PI3 kinase, phosphatidylinositol 3′-kinase; PDGF, platelet-derived growth factor; HPLC, high-pressure liquid chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide electrophoresis; PVDF, poly(vinylidene fluoride); TFA, trifluoroacetic acid; DMEM, Dulbecco's modified Eagle's medium.

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MATERIALS AND METHODS

p85 Plasmid Constructs. A *Bgl*II–*Eco*RV fragment was isolated containing the full-length coding sequence of mouse α-type p85 tagged with the 8 amino acid SV40 large T antigen KT3 epitope at the C-terminus and linked to the T7 $\phi 10$ promoter and terminator region (Klippel et al., 1992). This p85 expression cassette was ligated to *Bam*HI and blunted *Hin*dIII ends in the polylinker region of the bacterial expression/mutagenesis vector pMa (Stanssens et al., 1989) to generate pMa-85-KT3. Tyrosine to phenylalanine mutants of p85 were generated from pMa-85-KT3 by the gapped-duplex method (Stanssens et al., 1989) and then expressed in *Escherichia coli*. p85 sequences were also tagged with the 16 amino acid influenza hemagglutinin (IHA) epitope at the C-terminus and cloned into the mammalian expression vector pCG (pCG-85-IHA) as previously described (Klippel et al., 1993). Y508F mutant p85 sequences were cloned into pCG by subcloning a *Bsm*I–*Nsi*I fragment of pMa-Y508F-KT3 containing the mutated residue into pKS-85-IHA (Klippel et al., 1993) and then cloning the *Xba*I–*Bam*HI fragment of pKS-Y508F-IHA into pCG to generate pCG-Y508F-IHA.

Cell Culture. COS 6M cells were grown in DMEM containing 10% bovine calf serum, 50 μg/mL penicillin, and 50 μg/mL streptomycin to 50% confluency over 2–3 days before 10-cm plates were transfected with 5 μg of pCG-85-IHA or pCG-Y508F-IHA by the DEAE-dextran/chloroquine method (Sambrook et al., 1989). The medium was changed 24 h following transfection, and the cells were labeled with [^{32}P]orthophosphate after another 24 h (below).

Phosphorylation of p85 in Vitro. Recombinant wild-type or mutant p85 was expressed in *E. coli* and harvested by freeze–thawing in 50 mM Tris-HCl (pH 7.5), 50% sucrose, 5 mM EDTA, 5 mM EGTA, 0.25% Nonidet P-40, 2 mM dithiothreitol, and protease inhibitors as described in detail previously (Klippel et al., 1992). Recombinant PDGF β-receptors were expressed in baculovirus and harvested from Sf9 cells in cell lysis buffer containing 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM sodium orthovanadate, and protease inhibitors as described elsewhere (Summers & Smith, 1987; Morrison et al., 1989). p85 was phosphorylated as described previously by mixing lysates containing recombinant p85 and PDGF receptors in buffer containing 25 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 1 mCi of [γ-^{32}P]ATP (6000 Ci/mmol), 1 mM sodium orthovanadate, and protease inhibitors and incubating for 30 min at 30 °C (Kavanaugh et al., 1992). The reaction was terminated by the addition of EDTA to 5 mM and the p85 was then immunoprecipitated using polyclonal anti-p85 antisera (UBI) as described (Kavanaugh et al., 1992). The immunoprecipitates were then boiled in Laemmli sample buffer for 5 min.

Phosphorylation of p85 in Vivo. COS 6M cultures transfected with epitope-tagged p85 were washed with phosphate-free DMEM and then incubated in phosphate-free DMEM containing 1 mCi/mL [^{32}P]orthophosphate (ICN, 285 Ci/mg) for 4 h. The cultures were then treated with 2 nM BB-PDGF (C. G. Nascimento, Chiron Corp., Emeryville, CA) for 10 min at 37 °C. The cultures were then washed 3 times with ice-cold PBS and lysed at 4 °C in 1.0 mL of cell lysis buffer (above) per 10-cm plate. The lysates were cleared by centrifugation at 13000*g* for 10 min at 4 °C. Immunoprecipitations of COS 6M cells with the 12CA5 monoclonal anti-IHA antibody (Wilson et al., 1984) were performed as described (Kavanaugh et al., 1992).

Tryptic Phosphopeptide Analysis. Immunoprecipitated, ^{32}P-phosphorylated p85 (above) was analyzed by SDS–PAGE and transferred to a PVDF membrane (Immobilon, Millipore), and autoradiography was performed. The radioactive band corresponding to p85 was excised, washed thoroughly with water, and incubated with 1.5% poly(vinylpyrrolidone) 40 in 0.1 N acetic acid for 30 min at 37 °C. The membrane piece was then washed again with water, cut into approximately 1-mm pieces, and incubated with 250 μL of 20 μg/mL TPCK-treated trypsin (Worthington) in 50 mM NH_4HCO_3 overnight at 37 °C. Then an additional 250 μL of trypsin solution was added and the incubation was continued for another 6 h. The solution was transferred to a new tube and the membrane pieces were washed several times with water. The washes and trypsin solution were combined and used in two-dimensional phosphopeptide analysis or applied to HPLC, below.

Two-dimensional phosphopeptide analysis was performed as described (Boyle et al., 1991). The tryptic digest solution was vacuum-dried, resuspended in water, and redried several times to remove residual buffer. The samples were then resuspended in pH 1.9 buffer, and approximately equal amounts of radioactivity as determined by Cerenkov counting were spotted onto cellulose plates (EM Science) and subjected to thin-layer electrophoresis at pH 1.9 in one dimension and ascending thin-layer chromatography at pH 4.72 in the second dimension as described (Boyle et al., 1991). The plates were dried and exposed to X-ray film at −70 °C.

Alternatively, the tryptic phosphopeptides were separated by reverse-phase HPLC on a 2.1-mm × 25-cm C_{18} column (Vydac, Hesperia, CA). Peptides were eluted over 60 min with a 0–100% linear gradient of acetonitrile in 0.1% trifluoroacetic acid (TFA) at a flow rate of 0.5 mL/min. Fractions (250 μL) were collected at 0.5-min intervals and counted by Cerenkov counting. Radioactive peaks were then subjected to sequential cycles of Edman degradation using an Applied Biosystems 475 protein sequencer. The TFA extracts were analyzed by scintillation counting. Peptides containing phosphotyrosine (see text) were synthesized as described (Escobedo et al., 1991a) and applied to HPLC, and fractions were monitored by absorbance at 280 nm.

RESULTS

To map the tyrosine(s) on p85 which are phosphorylated by the PDGF receptor, recombinant mouse p85 was first phosphorylated *in vitro* with recombinant PDGF receptors. Lysate containing bacterially expressed p85 was mixed with lysate containing baculovirus-expressed, activated PDGF β-receptors in the presence of [γ-^{32}P]ATP and $MgCl_2$ as described in Materials and Methods. The phosphorylated p85 was then immunoprecipitated with polyclonal anti-p85 antisera and analyzed by SDS–PAGE and transfer to a PVDF membrane. Two bands were observed by autoradiography (Figure 1); the upper band represents autophosphorylated PDGF receptor, which binds tightly to p85 and therefore coimmunoprecipitates with it, and the lower band is p85. We have shown previously that under these conditions phosphorylation of p85 requires the presence of PDGF receptors and occurs on tyrosines (Kavanaugh et al., 1992). The 85-kDa band was then excised and digested with trypsin, and the phosphopeptides were purified by reverse-phase HPLC (Figure 2). Three major peaks of radioactivity, labeled A, B, and C in Figure 2, were consistently observed in repeated experiments. Occasionally, a smaller fourth peak (A′) and other minor peaks were observed (Figure 2). These may represent partial tryptic digestion products or peptides containing minor phosphorylation sites (see below).

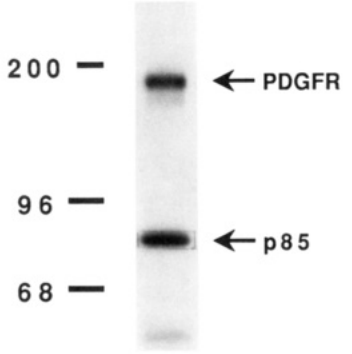

FIGURE 1: Phosphorylation of p85 by the PDGF receptor *in vitro*. Bacterially expressed p85 was tyrosine-phosphorylated by incubation with baculovirus-expressed PDGF receptor and [γ-^{32}P]ATP as described in Materials and Methods. p85 was then immunoprecipitated with polyclonal anti-p85 antisera, and analyzed by SDS–PAGE, transfer to a PVDF membrane, and autoradiography. A typical exposure time was 15 min. The position of the PDGF receptor, which coimmunoprecipitates with p85, is shown. Molecular mass markers are in kilodaltons.

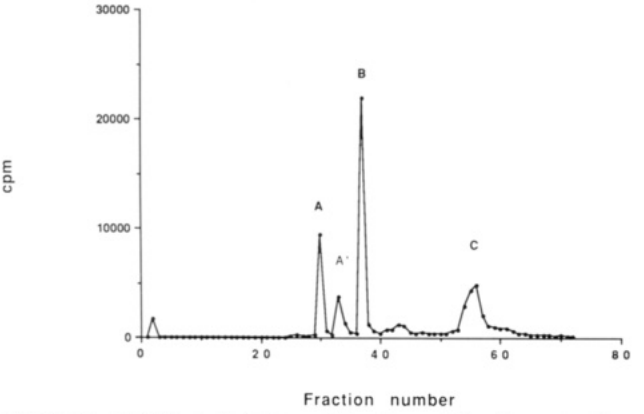

FIGURE 2: HPLC purification of tryptic peptides from *in vitro* phosphorylated p85. The p85 band seen in Figure 1 was excised and digested with trypsin as described in Materials and Methods. The tryptic products were applied to a reverse-phase HPLC column and eluted over 60 min with a 0–100% gradient of acetonitrile in 0.1% trifluoroacetic acid. Fractions (0.5 min, 250 μL) were collected and counted by Cerenkov counting. The peaks labeled A, B, and C were consistently observed in repeated experiments. A′ represents a minor peak seen in some experiments (see text).

The HPLC fractions containing the three major peaks of radioactivity were then subjected to sequential Edman degradation using an automated protein sequencer. In Edman degradation, amino acid residues in a peptide are derivatized, removed, and collected one at a time from the N-terminus (Boyle et al., 1991). By monitoring the radioactivity released at each cycle, the position of the phosphorylated residue relative to the N-terminus can be assigned. An elution profile of a Edman degradation run on peak A from p85 is shown in Figure 3 and demonstrates that a phosphotyrosine is present at the second residue from the N-terminus in this peptide. Although one might also assign a phosphorylated residue to position 10 on the basis of Figure 3, only the first radioactive peak in this type of analysis can be interpreted with confidence. Radioactivity leaching from the first phosphorylated residue can contaminate subsequent cycles in an unpredictable manner. Further, the assignment of a phosphotyrosine to position 10 was not confirmed in repeat experiments. Radioactivity was released at cycle 2 in two of three independent analyses of peak A, as well as in three of three analyses of peak B, indicating that both peaks A and B contain a phosphotyrosine at that position. An analysis of peak A′, which elutes from HPLC near peak A, revealed radioactivity at cycle 5. No phosphorylated residue could be unambiguously assigned in analysis of peak C.

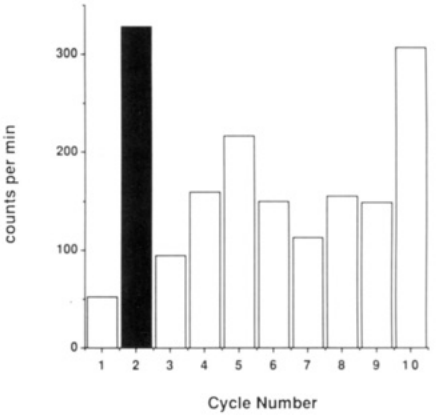

FIGURE 3: Edman degradation profile of a HPLC-purified tryptic phosphopeptide from p85. HPLC-purified tryptic phosphopeptides from *in vitro* phosphorylated p85 were subjected to Edman degradation on an automated protein sequencer, and the TFA extracts at each cycle were analyzed by scintillation counting. Shown is an elution profile of peak A (Figure 2). The first radioactive residue is shown in black.

Table 1: Predicted Tryptic Peptides from p85 Which Contain Tyrosine in the Second Edman Degradation Cycle

	predicted peptide	2nd cycle tyrosine
complete digest	^{462}EYDR^{465}	Y463
	^{466}LYEEYTR^{472}	Y467
	^{507}EYIGK^{511}	Y508
partial digest	^{188}RYLADLPNPVIPVAVYNEM-MSLAQELQSPEDCIQLLK^{224}	Y189

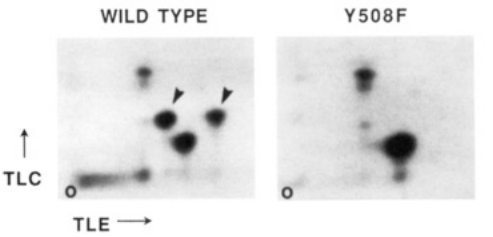

FIGURE 4: Two-dimensional tryptic phosphopeptide mapping of wild-type and Y508F mutant p85 phosphorylated *in vitro* by the PDGF receptor. Bacterially expressed wild-type and Y508F mutant p85 were phosphorylated by PDGF receptors as in Figure 1 and Materials and Methods. The excised p85 band was digested with trypsin and analyzed by thin-layer electrophoresis (TLE) at pH 1.9 in one dimension and by ascending thin-layer chromatography (TLC) at pH 4.72 in the second dimension, followed by autoradiography. The arrows indicate spots that disappear with the Y508F mutation. O designates the origin.

The above experiments demonstrated that p85 which had been tyrosine-phosphorylated *in vitro* by PDGF receptors contains a tryptic peptide(s) with a phosphotyrosine at the second residue relative to the N-terminus. Three peptides in the predicted complete tryptic digest of p85 contain tyrosines which occur in the second position: tyrosines 463, 467, and 508 (Table 1). Partial digestion with trypsin could produce a fourth peptide with tyrosine 189 at the second residue (Table 1). To determine which residue(s) were phosphorylation sites *in vitro*, tyrosines 463, 467, and 508 were mutated to phenylalanine. The proteins were then expressed in bacteria, phosphorylated with PDGF receptors as before, and subjected to two-dimensional tryptic phosphopeptide analysis. As shown in Figure 4, three major radioactive spots and several minor spots are seen in this analysis of phosphorylated wild-type

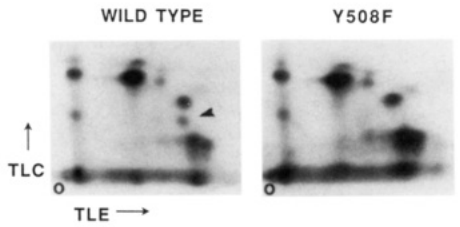

FIGURE 5: Y508 of p85 is phosphorylated *in vivo* in COS 6M cells. COS 6M cultures were transfected with either pCG-85-IHA (wild-type p85) or pCG-Y508F-IHA (mutant p85). Forty-eight hours later, the cultures were labeled with [^{32}P]orthophosphate and stimulated with PDGF, and p85 was immunoprecipitated from lysates with a monoclonal anti-IHA antibody (see Materials and Methods). The immunoprecipitates were then analyzed by two-dimensional tryptic phosphopeptide mapping as in Figure 4 and Materials and Methods. The arrow indicates the Y508-containing peptide. O designates the origin.

p85, consistent with the finding of three major peaks after HPLC of the same tryptic products. Mutation of tyrosines 463 and 467 to phenylalanine produced patterns identical with wild-type (data not shown). However, mutation of tyrosine 508 led to the disappearance of two of the major radioactive spots (Figure 4). This experiment demonstrates that tyrosine 508 of p85 is phosphorylated by the PDGF receptor *in vitro*.

The disappearance of two spots on two-dimensional phosphopeptide analysis of the Y508F mutation most likely results from incomplete digestion of the Y508 peptide by trypsin. This would be consistent with the observation that both peak A and peak B from HPLC contained a tyrosine at the same position by Edman degradation. To investigate this possibility and to confirm the results of the Edman degradation analysis, peptides derived from the p85 sequence were synthesized with phosphotyrosine at Y508 and analyzed by HPLC as before. A Y508-containing phosphopeptide corresponding to an incomplete tryptic digest product (E-pY^{508}-I-G-K-I-K) migrated at the position of peak B. Further, the phosphorylated residue in this synthetic phosphopeptides is at position 2, which is consistent with the Edman degradation analysis of peak B. These data are consistent with the hypothesis that peak B in Figure 2 (as well as one of the two spots indicated in Figure 4) is derived from incomplete digestion of the Y508 peptide by trypsin. Similar analysis suggests that peak A′ is also derived from incomplete tryptic digestion. Taken together, these results demonstrate that Y508 is the major phosphorylation site on p85 for the PDGF receptor *in vitro*.

To determine whether Y508 is phosphorylated *in vivo*, COS 6M cells were transiently transfected with either wild-type or Y508F mutant p85 tagged at the C-terminus with the influenza hemagglutinin (IHA) epitope. The COS cultures were then labeled with [^{32}P]orthophosphate, treated with PDGF, and lysed, and p85 was immunoprecipitated with a monoclonal anti-IHA antibody. The immunoprecipitates were then subjected to two-dimensional tryptic phosphopeptide analysis as before. As seen in Figure 5, a phosphopeptide present in tryptic maps of wild-type p85 phosphorylated *in vivo* is absent in maps of Y508F mutant p85 phosphorylated *in vivo*. This peptide contains phosphotyrosine and comigrates with one of the peptides identified in Figure 4 as containing phosphorylated Y508 (data not shown). These experiments demonstrate that Y508 is phosphorylated *in vivo* in COS cells. The remaining phosphopeptides seen in Figure 5 represent additional serine/threonine and tyrosine phosphorylation sites seen in COS cells.

DISCUSSION

Tyrosine phosphorylation of the 85-kDa subunit of PI3 kinase is likely to regulate its activity or its interaction with other signaling proteins involved in growth control (Kaplan et al., 1987; Carpenter et al., 1989; Hayashi et al., 1991, 1992, 1993; Kavanaugh et al., 1992; Soltoff et al., 1992). One approach to understanding the mechanisms by which this regulation might occur is to identify which of the 34 tyrosines on mouse p85-α are phosphorylated. In this report, we have combined tryptic phosphopeptide mapping, Edman degradation, and mutational analysis to demonstrate that tyrosine 508 of p85-α is the major phosphorylation site *in vitro* for PDGF receptors and is phosphorylated *in vivo*. The sequences surrounding Y508 (SKEYIGK) are consistent with proposed consensus sequences for tyrosine phosphorylation (Pearson & Kemp, 1991).

There exists at least one other tyrosine phosphorylation site on p85 which we were unable to determine. Tryptic phosphopeptide analysis of Y508F p85 (Figure 4) revealed at least two phosphopeptides which were unaffected by the mutation, and Edman degradation analysis of HPLC peak C (Figure 2) did not identify the phosphorylated residue in that peak (see Results). There are several possible explanations for this result. First, the recovery of radioactivity from [^{32}P]phosphotyrosine residues in the TFA extract during automated Edman degradation is inefficient. Therefore, phosphotyrosines in peptides with low specific radioactivity may not be detected. Similarly, broad HPLC peaks which contain mixtures of peptides, each with phosphorylated residues at different positions, may fail to produce discernible peaks. Finally, for technical reasons, Edman degradation was continued only for 10 cycles; therefore, any phosphorylated residue further than 10 amino acids from the N-terminus of the peptide would not be identified in these experiments.

We have tested the effect of the Y508F mutation on p85-associated PI3 kinase activity by transfecting mutant p85 tagged with the influenza hemagglutinin (IHA) epitope into COS cells or fibroblasts. Mutation of Y508 to phenylalanine did not affect the interaction of p85 with PI3 kinase activity when these assays were performed on anti-IHA immunoprecipitates (data not shown). One explanation for this result is that other residues may have to be phosphorylated either alone or in combination with Y508. As mentioned, there exists at least one additional tyrosine phosphorylation site for the PDGF receptor which we have not identified. Alternatively, Y508 may not participate in the regulation of PI3 kinase activity *per se* but rather in another, as yet undefined, aspect of p85 function. There is good reason to believe that p85 has other functions beside physically linking receptors to PI3 kinase. For example, p85 contains an SH3 domain, a domain with homology to bcr, and two SH2 domains (Escobedo et al., 1991b; Otsu et al., 1991; Skolnik et al., 1991). The SH3, bcr, and N-terminal SH2 domains are not necessary for interaction of p85 with the PDGF receptor, with the 110-kDa subunit of PI3 kinase, or with PI3 kinase activity (Klippel et al., 1992, 1993). As we have proposed previously, tyrosine phosphorylation of p85 could be involved in regulating the interaction of these domains with other protein(s) involved in PDGF signaling (Kavanaugh et al., 1992).

Hayashi et al. (1993) recently reported that Y368, Y580, and Y607 of bovine and human p85-α are phosphorylated by the insulin receptor. Y607 was the only tyrosine which was significantly phosphorylated *in vitro* by the insulin receptor, and no evidence was reported of phosphorylation of Y508 *in vivo* in response to insulin (Hayashi et al., 1993). In contrast,

we have demonstrated that Y508 is the major phosphorylation site for the PDGF receptor *in vitro* and is phosphorylated *in vivo*. Although phosphorylation of p85 by the insulin and PDGF receptors has not been compared directly, these data suggest that p85 is phosphorylated differently by the insulin and PDGF receptors. It is possible, however, that the PDGF and insulin receptors phosphorylate some common p85 residues. For example, Y607 lies 14 amino acids from the N-terminus of its predicted tryptic peptide and would not have been identified in our analysis (see Results). However, the observation that insulin and PDGF receptors may phosphorylate p85 differently suggests that p85 may be regulated differently by insulin and PDGF.

In conclusion, we have shown that Y508 of p85 is a phosphorylation site for the PDGF receptor. In the future, identification of other proteins which interact with p85 may help clarify the role of phosphorylation of Y508 in the regulation of PDGF signaling.

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