Modification of Phosphatidylinositol 3-Kinase SH2 Domain Binding Properties by Abl- or Lck-mediated Tyrosine Phosphorylation at Tyr-688*

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In cells expressing the oncogenic Bcr-Abl tyrosine kinase, the regulatory p85 subunit of phosphatidylinositol 3-kinase is phosphorylated on tyrosine residues. We report that this phosphorylation event is readily catalyzed by the Abl and Lck protein-tyrosine kinases in vitro, by Bcr-Abl or a catalytically activated Lck-Y505F in cotransfected COS cells, and by endogenous kinases in transfected Jurkat T cells upon triggering of their T cell antigen receptor. Using these systems, we have mapped a major phosphorylation site to Tyr-688 in the C-terminal SH2 domain of p85. Tyrosine phosphorylation of p85 in vivo or in vitro was not associated with detectable change in the enzymatic activity of the phosphatidylinositol 3-kinase heterodimer, but correlated with a strong reduction in the binding of some, but not all, phosphoproteins to the SH2 domains of p85. This provides an additional candidate to the list of SH2 domains regulated by tyrosine phosphorylation and may explain why association of phosphatidylinositol 3-kinase with some cellular ligands is transient or of lower stoichiometry than anticipated.

Phosphatidylinositol 3-kinases (PI3Ks) are a family of enzymes involved in a multiplicity of cellular functions, including cell proliferation and transformation (1–3), lymphocyte activation (4–7), G protein signaling (8), DNA repair (9), intracellular vesicle trafficking (10, 11), and inhibition of programmed cell death (12, 13). The currently best characterized type of PI3K is the heterodimeric enzymes that consist of a 110-kDa catalytic subunit (p110α or p110β; Refs. 14 and 15) and a 85-kDa regulatory subunit (p85α or p85β; Refs. 16–18), and that are utilized for signaling by activated growth factor receptors (3, 16) or other proteins having the general motif phosphotyrosine Tyr(P)-X-X-methionine (19), or, in some cases, Tyr(P)-X-X-leucine (20, 21). In T cells, the physiologically relevant ligands for p85 include tyrosine-phosphorylated CD28 (22), subunits of the T cell antigen receptor (20, 21, 23), CD5 (24), CD7 (25), and the c-Cbl proto-oncogene product (26). In addition to causing a subcellular relocation of PI3K, these SH2 ligands cause an allosteric activation of the catalytic p110 subunit, which is bound to the region between the two SH2 domains of p85 (15, 27–29).

Several additional modes of PI3K regulation have been demonstrated, and it is likely that they act in concert to regulate the production of 3-phosphorylated inositol phospholipids in response to a variety of stimuli. The catalytic p110 interacts with activated GTP-bound Ras proteins through a region adjacent to its p85-binding NH2 terminus (30, 31). Active Ras enhances PI3K activity in intact cells (30, 31), but some data indicate that Ras also acts downstream of PI3K (32). In T cells, two p85 isoforms have been shown to undergo phosphorylation on both serine and threonine (33, 34). Tyrosine phosphorylation of the p85 subunit has been shown to occur in many different systems, such as in response to platelet-derived growth factor (3), insulin (35), B cell antigen receptor ligation (4), interleukin-2 (36), and in cells transformed by the Bcr-Abl fusion protein-tyrosine kinase (37–40). The sites of phosphorylation in p85 have been mapped to tyrosines 368, 508, and 607 in insulin-stimulated cells (35), but the physiological function of this phosphorylation has remained unknown. Tyrosine phosphorylation of p85 seems not to be required for the enzymatic activity of PI3K. Instead, tyrosine phosphorylation of p85 has been reported to correlate with the dissociation of PI3K from the activated insulin receptor kinase (41).

We have studied the tyrosine phosphorylation of p85 in hematopoietic cells, and report that phosphorylation occurs at least at Tyr-688 in the C-terminal SH2 domain. This event does not detectably affect the catalytic activity of PI3K per se, but causes a change in the binding properties of the SH2 domain. This change is likely to modify the function of PI3K in intact cells.

MATERIALS AND METHODS

Antibodies and Reagents—Antibodies against p85 of PI3K and the anti-Tyr(P) mAb 4G10 were from Upstate Biotechnology Inc. (Lake Placid, NY) and the anti-HA epitope mAb 12CA5 was from Boehringer Mannheim (Indianapolis, IN). The hybridoma that produces the OKT3 anti-CD3ε mAb was from American Type Cell Collection. Phosphatidylinositol was from Upstate Biotechnology Inc. and TPCK-treated trypsin was purchased from Promega Corp.
sin was from Worthington Biochemicals (NJ). Recombinant Abl protein-tyrosine kinase domain (Abl-K) was from New England Biolabs (Beverly, MA) and purified Lck from Upstate Biotechnology Inc. The protein-tyrosine kinase expression plasmids have been used (42, 43), and we recently described the cloning of the HA-tagged p85 construct (15). In addition to an N-terminal hemagglutinin (HA) tag, the constructs contain the following amino acids of bovine p85: N-SH2 (329–439), C-SH2 (563–724), NC (329–439 + 563–724), Nic (329–724), p85ΔSH2 (1–439 + 563–724), and wide-type p85 (1–724). GST fusion proteins containing the same fragments of p85 were ligated into the glutathione S-transferase (GST) fusion vector pGEX-4T-2 (Pharmacia, Sweden) by standard procedures. Expression was induced and the fusion proteins were purified using glutathione-Sepharose beads (Pharmacia).

**Cells**—Jurkat T leukemia cells, and their simian leukemia virus 40 large T antigen-expressing variant, J-TAg (kind gift from Dr. M. Karin), were kept at logarithmic growth in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, l-glutamine, and antibiotics. HL-60, Bcr-Abl cells were obtained by retroviral transfection of HL-60 promyelocytic leukemia cells with pShoSvMSVp185-avr-tkneo as described elsewhere (37). COS-1 cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum.

**Transient Transfections**—15 × 10⁶ J-TAg cells, HL-60, or HL-60/Bcr-Abl were transfected with a total of 10–20 μg of DNA by electroporation at 800 microfarads and 240 V. Typically, cells were transfected with 15 μg of HA-tagged p85 construct. Empty vector was added to control samples to make a constant amount of DNA in each sample. Cells were harvested two days after electroporation. COS-1 cells were transfected by lipofection with 10 μg of DNA and grown for 48 h prior to the experiments as described (42, 44–47).

**Immunoprecipitation**—These procedures were as reported before (5, 42, 48, 47). All steps were carried out at 0–4 °C. Cells were lysed in 20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA containing 1% Nonidet P-40, 1 mM Na₃VO₄, 10 μg/ml aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 100 μg/ml soybean trypsin inhibitor and clarified by centrifugation at 13,000 × g for 10 min. The clarified lysates were preabsorbed on agarose-conjugated goat anti-rabbit IgG or protein G-Sepharose. The lysates were then incubated with antibody for 2–4 h, followed by agarose-conjugated goat anti-rabbit IgG or protein G-Sepharose. Immune complexes were washed three times with lysis buffer, once in lysis buffer with 0.5 mM NaCl, again in lysis buffer, and either suspended in SDS sample buffer or washed further for kinase assays.

**SDS-PAGE and Western Blotting**—Proteins were separated by SDS-PAGE and transferred onto nitrocellulose filters. The antibodies were used at 1:500–1:2000 dilution and the blots developed by the enhanced chemiluminescence technique (ECL kit, Amersham) according to the manufacturer’s instructions.

**In Vitro Phosphorylation of GST Fusion Proteins**—The GST fusion proteins were dissolved in kinase buffer (10 mM HEPES pH 7.5, 0.1% Triton X-100, 20 mM MgCl₂, 1 mM dithiothreitol, and 0.1 mM Na₃VO₄). After preincubation of 2 mM cold ATP or 1 μM ATP and 10 μCi of [γ-³²P]ATP and 1 μl of purified Lck or 100 units of Abl-K, the reaction was carried out for 30 min at 30 °C or overnight at room temperature (for higher stoichiometry of phosphorylation). The reaction was stopped by adding SDS sample buffer or by adding cold lysis buffer. In the latter case, the mixture was incubated with glutathione-Sepharose beads for 1 h, and the beads washed extensively before they were added to preclared cellular lysates.

**Binding of Cellular Proteins to GST Fusion Proteins**—These experiments were done as before (48). Cell lysates were prepared as above, cleared by centrifugation, and preadsorbed to glutathione-Sepharose beads. After removal of the beads, the lysates were incubated on ice with 5 μg of GST fusion protein and glutathione-Sepharose beads for 2 h, which were subsequently washed five times with lysis buffer. The bound proteins were eluted in SDS sample buffer, resolved by SDS-PAGE, and analyzed by immunoblotting as above.

**P38 Assay**—The assay for lipid kinase activity of immunoprecipitated P38K was as before (5, 6, 49). The assay mixture contained 20 mM Tris/HCl, pH 7.5, 100 mM NaCl, 0.5 mM EGTA, 10 μg of phosphatidylinositol (stock solution at 20 mg/ml in water), 20 mM MgCl₂, and 10 μCi of [γ-³²P]ATP. Reaction products were analyzed by ascending chromatography in Silica gel thin layer plates in CHCl₃, CH₃OH, 25% NH₄OH/H₂O (90:90:9:19) followed by autoradiography.

**Tryptic Peptide Mapping**—GST-p85-NIC protein phosphorylated by Lck in the presence of [γ-³²P]ATP was resolved on 10% SDS gels, transferred onto a nitrocellulose filter, exposed to film, and the correct band excised. The filter piece was blocked and digested with TPCK-

**RESULTS**

**Tyrosine Phosphorylation of p85 in Bcr-Abl-expressing HL-60 Cells**—Bcr-Abl induces transformation of fibroblasts and hematopoietic cells (51–54) and confers resistance to apoptosis (55). Cells expressing the Bcr-Abl tyrosine kinase contain elevated amounts of proteins phosphorylated on tyrosine residues. At least in some Bcr-Abl expressing cells, HL-60 cells transfected with Bcr-Abl (37), a fraction of the p85 subunit is included among these substrates for enhanced tyrosine phosphorylation. When P38K was immunoprecipitated from parental HL-60 cells with antibodies against the p85 subunit and immunoblotted with anti-Tyr(P) mAbs, no phosphorylation of p85 could be detected (Fig. 1, lane 1), even if the cells were pretreated with 100 μM pervanadate to increase intracellular Tyr(P) content (lanes 2 and 3). In contrast, when anti-p85 immunoprecipitates prepared from HL-60 cells stably transfected with Bcr-Abl (37), a fraction of the p85 subunit is included among these substrates for enhanced tyrosine phosphorylation. When P38K was immunoprecipitated from parental HL-60 cells with antibodies against the p85 subunit and immunoblotted with anti-Tyr(P) mAbs, no phosphorylation of p85 could be detected (Fig. 1, lane 1), even if the cells were pretreated with 100 μM pervanadate to increase intracellular Tyr(P) content (lanes 2 and 3). In contrast, when anti-p85 immunoprecipitates prepared from HL-60 cells stably transfected with Bcr-Abl (HL-60/Bcr-Abl cells) were analyzed in parallel, a sharp, but not very prominent, band at 85 kDa (in addition to several other phosphoproteins) was seen (Fig. 1, lane 4). Reprobing of the same filter with anti-p85 revealed that all immunoprecipitates contained equal amounts of p85, which co-migrated precisely with the Tyr(P)-containing 85-kDa band in lane 4. This result was obtained in several independent experiments.

**Tyrosine Phosphorylation of p85 Does Not Affect P38K Activity**—To investigate whether this low level tyrosine phosphorylation of p85 in HL-60/Bcr-Abl cells has any effect on the catalytic activity of P38K, we first immunoprecipitated P38K from resting Jurkat T cells, and phosphorylated the immune com-
Regulation of p85 SH2 Domain

Phosphorylation of p85 in vitro or in vivo does not activate PI3K. Upper panels, anti-Tyr(P) immunoblot of anti-p85 immunoprecipitates from Jurkat T cells left untreated (lane 1), or treated with recombinant Abl-K kinase domain (lane 2), or of anti-Tyr(P) immunoprecipitates (lanes 3 and 4) or anti-p85 immunoprecipitates (lanes 5 and 6) from parental HL-60 (lanes 3 and 5) or HL-60 cells transfected with Bcr-Abl (lanes 4 and 6). Lower panels, anti-p85 immunoblot of the same filter. The migration of p85 is indicated. The right-hand panel shows a lipid kinase assay of the same immunoprecipitates. Note that the lipid kinase activity correlates very closely with the amount of p85 in the immunoprecipitates.

To further study the effect of in vivo phosphorylation on PI3K activity, we assayed anti-Tyr(P) and anti-p85 immunoprecipitates from wild-type HL-60 and HL-60/Bcr-Abl cells for PI3K activity. The anti-Tyr(P) immunoprecipitates contained 5-fold higher PI3K activity from HL-60/Bcr-Abl cells than from parental HL-60 cells (Fig. 2, right, lanes 3 and 4). This increase was accompanied by a similar increase in the amount of p85 protein present in these immunoprecipitates (Fig. 2, center, lower panel, lanes 3 and 4). Anti-p85 immunoprecipitates from both cell types contained equal amounts of p85 protein (Fig. 2, center, lower panel, lanes 5 and 6), but an anti-Tyr(P) immunoblot of the same filter revealed that p85 from wild-type HL-60 cells was completely unphosphorylated while p85 and several co-immunoprecipitated proteins were heavily tyrosine phosphorylated from Bcr-Abl expressing cells (Fig. 2, center, lanes 5 and 6). Despite this difference, the PI3K activity in these immunoprecipitates was similar in both cases. In some experiments, there was a marginal increase in PI3K activity in anti-p85 immunoprecipitates from Bcr-Abl-expressing cells, probably due to the binding of phosphoproteins to the SH2 domains of p85, as seen in the anti-Tyr(P) blot.

We conclude from these experiments that PI3K activity correlated closely with the amount of p85 protein regardless of its phosphorylation status. Apparently, tyrosine phosphorylation of p85 is not required for enzymatic activity and at the stoichiometry that it occurs in intact cells it does not directly affect the enzymatic activity of PI3K to any measurable degree.

Bcr-Abl Phosphorylates p85 in Its SH2 Domains—To identify in which part of the molecule p85 is tyrosine phosphorylated in HL-60/Bcr-Abl cells, we transiently transfected these cells with a set of HA-tagged truncated p85 constructs, which were subsequently immunoprecipitated and analyzed by anti-Tyr(P) immunoblotting. In these experiments (Fig. 3), wild-type p85 was phosphorylated on tyrosine, as was p85 lacking the inter-SH2 domain (p85ΔISH2) and the two constructs containing both SH2 domains with or without the inter-SH2-domain (NIC and NC, respectively). In contrast, the individual SH2 domain proteins (N-SH2 and C-SH2) did not contain detectable Tyr(P) even on very long exposures. All constructs were expressed at comparable levels as judged by anti-HA tag immunoblotting (Fig. 3, lower panel). Since the N-SH2 and C-SH2 proteins together contain the same amino acids as the NC protein, we conclude that the main phosphorylation occurs in one or both of these two domains, but that both are required for phosphorylation in intact HL-60/Bcr-Abl cells. This requirement correlates with the co-immunoprecipitation of several cellular phosphoproteins with all constructs having both SH2 domains (Fig. 3), but not the single SH2 domains. Thus, proper interaction of p85 with other proteins, perhaps including Bcr-Abl itself, is important for efficient phosphorylation.

Activated Lck Phosphorylates p85 in the C-terminal SH2 Domain—We have previously used a transient COS-1 cell transfection system to study potential interactions between protein-tyrosine kinases and the role of phosphorylation sites (42, 44–47). When full-length p85 or its truncated versions were expressed in these cells together with an activated (56–58) Y505F-mutated Lck, we observed that all constructs having two SH2 domains were efficiently phosphorylated in these cells.
FIG. 4. Tyrosine phosphorylation of p85 constructs by Lck-Y505F in COS cells. Upper panels, anti-Tyr(P) immunoblot of anti-HA tag immunoprecipitate from COS cells transfected with empty pEF/HA vector (lane 1), p85ΔiSH2 (lanes 2 and 3), C-SH2 (lanes 4 and 5), or N-SH2 (lanes 6–10) alone or together with Lck-Y505F (lanes 3, 5, and 7), Bcr-Abl (lane 8), Bmx (lane 9) or Jak2 (lane 10). Lower panels, anti-HA tag immunoblot of the same filter. The migration of the constructs and Ig heavy and light chains are indicated.

FIG. 5. Tyrosine phosphorylation of p85-NC by a TCR-activated protein-tyrosine kinase PTK in T cells. Upper panel, anti-Tyr(P) immunoblot of anti-HA tag immunoprecipitate from J-TAg cells transfected with empty pEF/HA vector (lanes 1–5), or p85-NC (lanes 6–10), and left untreated (lanes 1 and 6) or treated with OKT3 for 1 min (lanes 2 and 7), 3 min (lanes 3 and 8), 5 min (lanes 4 and 9), or 10 min (lanes 5 and 10). Lower panel, anti-HA tag immunoblot of the same filter. The migration of NC and Ig heavy and light chains are indicated. In the upper panel, the migration of three major phosphoproteins is also indicated.

phorylated with time (Fig. 5). Peak tyrosine phosphorylation occurred at the 5-min time point. The NC protein was equally immunoprecipitated in each sample (lower panel), and it also co-precipitated a set of cellular phosphoproteins at 36–38, 70, and 120 kDa. These proteins are similar in size to those that co-immunoprecipitate with endogenous p85 (5, 49) or that bind to GST fusion proteins of the two SH2 domains of p85 (see below).

Both Abl and Lck Phosphorylate the C-terminal SH2 Domain of p85 in Vitro—As phosphorylation of the C-SH2 protein was relatively low in intact cells, perhaps due to less efficient recruitment or inappropriate subcellular location, we decided to test if Abl-K and Lck would display specificity for this region of p85 in vitro. GST fusion proteins were prepared of the N-SH2, C-SH2, NC, and NiC constructs, and these proteins (or control GST) were incubated with recombinant Abl-K or purified Lck in the presence of \([\gamma^{32}P]\)ATP and analyzed the reaction product. As shown in Fig. 6, all fusion proteins, except GST-N-SH2, were well and equally phosphorylated. The GST-C-SH2 was close in size to Abl-K making its phosphorylation difficult to appreciate in lane 5, but the size of Lck was larger and the phosphorylation of GST-C-SH2 is easily seen in lane 6. Control GST was very weakly phosphorylated by Abl-K or Lck, indicating that the phosphorylation of the p85 protein occurred in the p85-derived parts. Similar results were obtained in several independent experiments.

Mapping of the Major Phosphorylation Site in the SH2 Domains of p85 to Tyr-688—to determine how many tyrosine residues were phosphorylated in the C-terminal SH2 domain of p85 in vitro, we phosphorylated the GST-NiC protein by Abl-K in the presence of \([\gamma^{32}P]\)ATP and analyzed the reaction product by tryptic peptide mapping. As shown in the bottom panel of Fig. 6, the protein was phosphorylated at one major site (peptide a), a few minor sites and a variable site (peptide b), which may be derived from the GST part of the fusion protein as peptides with similar mobility can be seen in other in vitro
phosphorylated GST fusion proteins (46). A similar map was obtained when GST-NiC was phosphorylated with Lck (not shown). Since the maps contained only one major peptide, we decided to map the site through site-directed mutagenesis of a construct from a lysate of pervanadate-treated Jurkat T cells. Before mixing with the lysate, the GST fusion protein was pretreated for 12 h at room temperature with buffer alone (lane 1), with buffer and 2 mM ATP (lane 2), or with buffer, ATP, and 100 units of Abl-K (lane 3). The lower panel, anti-GST blot of the same filter. The right hand panels of experiment 1 are an anti-Cbl blot (top), anti-Zap blot (middle), and anti-TCRζ blot (bottom) of the same filter. In experiment 2, the experiment was carried out similarly, but the GST-NC protein was also treated with purified Lck (lane 4). Shown is the anti-Tyr(P) blot (top), and anti-TCRζ blot (bottom).

The location of Tyr-688 in the C-terminal SH2 domain—The location of Tyr-688 in the C-terminal SH2 domain of p85 NC is Tyr-688 in the C-terminal SH2 domain. This site is an important target for kinases such as Abl and Lck, which are known to phosphorylate this residue in vitro and in vivo. The phosphorylation of Tyr-688 by Abl and Lck has been shown to modulate the binding properties of the p85 SH2 domain.

Fig. 6. Tyrosine phosphorylation of p85 domains in vitro. Upper panel, anti-Tyr(P) immunoblot of the indicated GST fusion proteins incubated alone (lanes 1, 4, 7, 10, and 13), with recombinant Abl kinase domain (lanes 2, 5, 8, 11, and 14) or with purified Lck (lanes 3, 6, 9, 12, and 15) in the presence of 1 mM ATP. Note that Abl-K migrates very close to GST-C-SH2 and Lck as GST-NC. Middle panel, anti-GST blot of the same filter. Lower panel, tryptic peptide maps of wild-type, Y607F, Y685F, or Y688F mutated GST-NiC phosphorylated by Abl-K in the presence of γ-32P]ATP. Peptide a was not seen in maps of GST, but peptide b is variably present in the maps of other GST fusion proteins phosphorylated in vitro.

Fig. 7. Tyrosine phosphorylation of p85-NC by Lck or Abl reduces its ligand binding capacity. Two separate experiments are shown. In experiment 1, the upper left panel is an anti-Tyr(P) immunoblot of material bound to the GST-NC construct from a lysate of pervanadate-treated Jurkat T cells. Before mixing with the lysate, the GST fusion protein was pretreated for 12 h at room temperature with buffer alone (lane 1), with buffer and 2 mM ATP (lane 2), or with buffer, ATP, and 100 units of Abl-K (lane 3). The lower panel, anti-GST blot of the same filter. The right hand panels of experiment 1 are an anti-Cbl blot (top), anti-Zap blot (middle), and anti-TCRζ blot (bottom) of the same filter. In experiment 2, the experiment was carried out similarly, but the GST-NC protein was also treated with purified Lck (lane 4). Shown is the anti-Tyr(P) blot (top), and anti-TCRζ blot (bottom).

The size of the 21- and 70-kDa proteins, and their absence in the EF loop, Tyr-192, causes a dramatic decrease in its binding of ligands (47). To test this possibility in the case of the p85 SH2 domains, we incubated the GST-NC protein with or without ATP and Abl-K overnight at room temperature to obtain as high stoichiometry of phosphorylation as possible. Following this incubation, the GST fusion protein was adsorbed to glutathione-Sepharose, washed extensively, and mixed with a pre-cleared lysate of pervanadate-treated Jurkat cells. After 2 h on ice, the Sepharose beads were again washed extensively and bound proteins eluted in SDS sample buffer, resolved on SDS gels, transferred onto nitrocellulose filters, and analyzed by anti-Tyr(P) immunoblotting. These experiments revealed that preincubation of the GST fusion protein with Abl-K and ATP, but not ATP alone, caused several significant changes to the set of cellular phosphoproteins subsequently bound to the SH2 domains of p85 (Fig. 7). In particular, a band at 21 kDa and another at 70 kDa decreased very markedly, while other bands remained unchanged or even increased. An anti-GST blot showed that the loading of GST fusion protein was equal in all samples. The band at ~65 kDa contains the GST-NC itself, and its phosphorylation can be seen in lanes 3 and 4. This result was obtained in several independent experiments, two of which are shown in Fig. 7. In other experiments we obtained similar results with lysates from Jurkat cells treated with anti-CD3ε instead of pervanadate (not shown). In the other experiment shown, a similar result was obtained with preincubation of GST-NC with Lck (lane 4) in addition to incubation with Abl-K (lane 3).

The size of the 21- and 70-kDa proteins, and their absence in experiments using HL-60 cells (not shown), prompted us to blot the samples also with antibodies specific for TCRζ and Zap-70 (64), both of which were found to be present, and the binding of both decreased clearly upon tyrosine phosphorylation of the GST-NC protein. In contrast, the c-Cbl protein was also pre-
ent, but bound equally well to unphosphorylated as well as tyrosine-phosphorylated GST-NC. Perhaps this protein binds well to the N-terminal SH2 domain, which may not be affected by phosphorylation of the C-terminal SH2 domain. Attempts to perform these experiments with the GST-C-SH2 protein (instead of GST-NC) were not successful due to the low binding of cellular proteins to this isolated domain.

**DISCUSSION**

Taken together, our findings indicate that PI3K can be phosphorylated at Tyr-688 in the C-terminal SH2 domain of the p85 subunit in Bcr-Abl expressing HL-60 cells, by active Lck in COS cells, by an unidentified receptor-activated protein-tyrosine kinase in T cells, and by both Abl and Lck in *vivo*. This phosphorylation does not measurably affect the lipid kinase activity of PI3K (at least at physiological stoichiometry), but was found to change the ligand binding properties of the SH2 domain(s). These results are in agreement with previous observations showing that expression of Bcr-Abl in NIH 3T3 cells induces tyrosine phosphorylation of PI3K without any significant increase in PI3K products *in vivo* (40).

The analysis of SH2 domain function in p85 is complicated by the tandem arrangement of the two SH2 domains, resulting in their cooperative binding to many ligands. Our results indicate that tyrosine phosphorylation of the C-terminal SH2 domain reduced the affinity for some ligands, while the binding of others was unchanged. Two alternative explanations could be envisioned: either tyrosine phosphorylation changed the ligand selection from the classical Tyr(P)-X-X-metotopes to something more or less different, or only the C-terminal SH2 domain was inhibited, while the N-terminal SH2 domain remained unchanged. In the latter case, only of those ligands that bind exclusively to the C-terminal SH2 domain or that require simultaneous binding to both domains will bind less strongly.

We have previously observed that expression of the HA-tagged p85 proteins in T cells resulted in the co-immunoprecipitation of phospho-TCRζ only when both SH2 domains were present in the p85 protein. Such a requirement for two SH2 domains would explain why the TCRζ can bind the two SH2 domains of p85 simultaneously and thereby increase the affinity for many ligands, while the binding of others was unchanged. Two alternative explanations could be envisioned: either tyrosine phosphorylation changed the ligand selection from the classical Tyr(P)-X-X-metotopes to something more or less different, or only the C-terminal SH2 domain was inhibited, while the N-terminal SH2 domain remained unchanged. In the latter case, only of those ligands that bind exclusively to the C-terminal SH2 domain or that require simultaneous binding to both domains will bind less strongly.

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