Regulation of the p85/p110α Phosphatidylinositol 3′-Kinase
DISTINCT ROLES FOR THE N-TERMINAL AND C-TERMINAL SH2 DOMAINS

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Our previous studies on the p85/p110α phosphatidylinositol 3-kinase showed that the p85 regulatory subunit inhibits the p110α catalytic subunit, and that phosphopeptide activation of p85/p110α dimers reflects a disinhibition of p110α. Phosphopeptide binding to the nSH2 domain mediates both inhibition of p110α and disinhibition by phosphopeptides. In contrast, phosphopeptides did not increase the activity of iSH2/cSH2-p110α dimers, or dimers composed of p110α and an nSH2/iSH2/cSH2 construct containing a mutant nSH2 domain. Phosphopeptide binding to the cSH2 domain increased p110α activity only in the context of an intact p85 containing both the nSH2 domain and residues 1–322 (the SH3, proline-rich and breakpoint cluster region-homology domains). These data suggest that the nSH2 domain of p85 is a direct regulator of p110α activity. Regulation of p110α by phosphopeptide binding to the cSH2 domain occurs by a mechanism that requires the additional presence of the nSH2 domain and residues 1–322 of p85.

PI 3′-kinases form a diverse family of lipid kinases that phosphorylate phosphatidylinositol at the D3-position (1). The regulation of the p85/p110 PI 3′-kinase is particularly complex. The p85 regulatory subunit contains an N-terminal SH3 domain followed by a proline rich domain, a breakpoint cluster region-homology domain, a second proline-rich domain, and two SH2 domains linked by a putative coiled coil domain (the inter-SH2 or iSH2 domain) that binds to the N terminus of the p110α catalytic subunit (2–4). The binding of proteins such as the p85 regulatory subunit to the p110 catalytic subunit, and that phosphatidylinositol 3-kinase showed that the p85 regulatory subunit inhibits the p110α catalytic subunit, and that phosphopeptide activation of p85/p110α dimers reflects a disinhibition of p110α. Phosphopeptide binding to the nSH2 domain mediates both inhibition of p110α and disinhibition by phosphopeptides. In contrast, phosphopeptides did not increase the activity of iSH2/cSH2-p110α dimers, or dimers composed of p110α and an nSH2/iSH2/cSH2 construct containing a mutant nSH2 domain. Phosphopeptide binding to the cSH2 domain increased p110α activity only in the context of an intact p85 containing both the nSH2 domain and residues 1–322 (the SH3, proline-rich and breakpoint cluster region-homology domains). These data suggest that the nSH2 domain of p85 is a direct regulator of p110α activity. Regulation of p110α by phosphopeptide binding to the cSH2 domain occurs by a mechanism that requires the additional presence of the nSH2 domain and residues 1–322 of p85.

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subcloned into pGEX-p85 to produce pGEX-p85-R358A and pGEX-p85-R659A. All mutations were produced with the Quick-Change mutagenesis kit and confirmed by sequencing.

Production of Recombinant Proteins—N-terminal Myc-tagged p110α was produced in baculovirus-infected SF-9 cells as described previously (11). Recombinant GST-fusion proteins were produced in Escherichia coli strain BL-21 and isolated on glutathione-Sepharose beads (Amersham Pharmacia Biotech). GST-fusion proteins were eluted with 10 mM glutathione, dialyzed into phosphate-buffered saline, and stored in 50% glycerol at −20 °C. Alternatively, bead-bound fusion proteins were washed into 50 mM ammonium bicarbonate, pH 7.8, cleaved with thrombin (25 units in 2 μl), lyophilized and resuspended in 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, and phenylmethylsulfonyl fluoride (350 μg/ml).

p110α Binding Assay—Recombinant N-myc-p110α was absorbed onto anti-Myc (Oncogene Science)-Protein G beads. N-myc-p110α beads or control beads were washed 3 times in 10 mM Tris, pH 7.5, 100 mM sulfonyl fluoride (350 μg/ml) for 1 h at 4 °C. After 3 more washes, absorbed proteins were analyzed by Western blotting with anti-iSH2 antibodies (13) followed by 125I-Protein A. Alternatively, GST-fusion proteins were immobilized on glutathione-Sepharose, washed, and incubated with Nonidet P-40 lysates from HEK 293T cells transfected with myc-p110α (20 μg DNA/10 cm dish, lysed 48 h after transfection). The samples were washed and absorbed proteins were analyzed by blotting with monoclonal anti-myc antibodies (Oncogene Science)/rabbit anti-mouse secondary antibodies followed by 125I-Protein A, and reblotting with rabbit anti-GST antibodies.

p110α Inhibition and Activation Assays—35 μl of lysate from control SF-9 or SF-9 cells expressing N-myc-p110α were incubated with 5 μg of recombinant p85 or p85 fragment for 1 h at 4 °C. The mixtures were then assayed directly for PI 3-kinase activity using sonicated phosphatidylinositol as a substrate as described previously (11). Alternatively, mixtures of p110α and p85 or fragments of p85 were incubated in the absence or presence of a bisphosphotyrosyl peptide derived from the Tyr-608/Tyr-628 region of IRS-1 (1) in the absence or presence of varying concentrations of unlabeled bisphosphotyrosyl peptide. The data are expressed as stimulation over the activity of each mixture in the absence of phosphopeptide. The data are the mean ± S.E. from four experiments.

RESULTS

The C-terminal Half of p85 Is Sufficient for Inhibition of p110α—p110α is maximally active as a monomer, and is inhibited by dimerization with p85 (11). To determine the domains of p85 required for this inhibition, we compared the activity of p110α when reconstituted with a fragment of p85 containing the C-terminal half of p85 (nSH2/iSH2-cSH2), or the iSH2 domain of p85 alone (Fig. 1A). Work from several laboratories has identified the iSH2 domain of p85 as the region that binds to p110α (14–17). Consistent with these observations, both the nSH2-iSH2-cSH2 and iSH2 fragments of p85 bound to immobilized p110α beads, but not to control beads (Fig. 1B). Incubation of p110α with the nSH2-iSH2-cSH2 fragment inhibited its activity by approximately 80% (Fig. 1C, lane b), and subsequent incubation of bisphosphotyrosyl peptide with either the p85/p110α or nSH2-iSH2-cSH2/p110α dimers increased their activity by 100% (Fig. 1D, lanes a and b). Surprisingly, the iSH2 domain could bind p110α (Fig. 1B) but had no effect on its activity (Fig. 1C, lane c). These data show that binding by the iSH2 domain to p110α is not sufficient to inhibit its catalytic activity, and suggest that the SH2 domains exert an additional constraint on p110α activity.

Inhibition of p110α by Single SH2-iSH2 Fragments—Dhand et al. (14) predicted that the iSH2 domain of p85 should exist as two antiparallel helices, which would place the SH2 domains of p85 is close apposition. It is possible that the two SH2 domains are close enough contact each other, thereby imposing a strained conformation of the iSH2 domain. This strain would be relieved by conformational changes in either SH2 domain upon phosphopeptide binding. Alternatively, each SH2 domain could independently affect the conformation of the iSH2 domain upon phosphopeptide binding. Alternatively, each SH2 domain could independently affect the conformation of the iSH2 domain upon phosphopeptide binding. It is likely that the two SH2 domains are close enough contact each other, thereby imposing a strained conformation of the iSH2 domain. This strain would be relieved by conformational changes in either SH2 domain upon phosphopeptide binding. Alternatively, each SH2 domain could independently affect the conformation of the iSH2 domain upon phosphopeptide binding. Alternatively, each SH2 domain could independently affect the conformation of the iSH2 domain upon phosphopeptide binding.

Regulation of p85/p110α PI 3'-Kinase

FIG. 1. The iSH2 domain is sufficient to bind but not inhibit p110α. A, nSH2/iSH2/cSH2 and iSH2 structures. B, recombinant N-myc-p110α was immobilized on anti-myc/Protein G beads. Control beads (lanes c and d) or p110α beads (lanes a and b) were incubated with recombinant iSH2 domain (lanes a and c) or nSH2/iSH2/cSH2 (lanes b and d). The proteins were eluted and separated by SDS-polyacrylamide gel electrophoresis, and visualized by Western blotting with anti-iSH2 antibodies and [125I]protein A. The data is representative of two experiments. C, recombinant N-myc-p110α was incubated in the absence or presence of recombinant nSH2/iSH2/cSH2 or iSH2. The mixtures were then assayed for PI 3'-kinase activity, and expressed as percent of p110α alone. The data are the mean ± S.E. from four experiments. D, the mixtures were incubated for an additional hour in the absence or presence of 1 μM bisphosphotyrosyl peptide, and PI 3'-kinase kinase activity was measured. The data are expressed as stimulation over the activity of each mixture in the absence of phosphopeptide. The data are the mean ± S.E. from four experiments.
Surprisingly, dimerization of p110α with the iSH2/cSH2 fragment was only slight inhibitory (Fig. 2C, lane d), and no increase in activity was seen in the presence of tyrosine phosphopeptide (Fig. 2D, lane c). These data suggest that the C-terminal SH2 domain contributes little to the inhibition of p110α, which is primarily because of the N-terminal SH2 domain.

Role of individual SH2 domains in phosphopeptide regulation of p110α/nSH2-iSH2-cSH2 dimers—We have previously shown that in dimers containing p110α and intact p85, both SH2 domains contribute to the increase in activity caused by tyrosine phosphopeptides (10). We therefore examined the activity of p110α when bound to nSH2-iSH2-cSH2 constructs containing disabling point mutations in the conserved FLVRES motifs of the N-terminal or C-terminal SH2 domains (R358A and R659A).

Fig. 2. Inhibition of p110α by the iSH2 domain linked to single SH2 domains. A, nSH2/iSH2/cSH2, nSH2/iSH2 and iSH2/cSH2 structures. B, left panel: control beads or myc-p110α beads were incubated with recombinant nSH2/iSH2 or iSH2/cSH2 (10 μg) as in Fig. 1, and bound proteins were analyzed by blotting with anti-iSH2 antibodies; right panel: control glutathione-Sepharose beads or beads containing immobilized GST-nSH2/iSH2 or GST-iSH2/cSH2 (5 μg) were incubated with lysates from HEK 293T cells expressing myc-p110α. Bound proteins were analyzed by sequential blotting with anti-myc and anti-GST antibodies.

Fig. 3. Inhibition of p110α by the nSH2/iSH2/cSH2 constructs containing mutant SH2 domains. A, nSH2-iSH2-cSH2, nSH2-R358A/iSH2/cSH2 (R358A), or nSH2/iSH2-cSH2-R659A (R659A) structures. B, p110α was incubated for 30 min at 4 °C with GST, nSH2/iSH2/cSH2, nSH2-R358A/iSH2/cSH2 (R358A), or nSH2/iSH2-cSH2-R659A (R659A). The mixtures were then assayed for PI 3'-kinase activity, and expressed as % of p110α alone. The data are the mean ± S.E. from three experiments. C, the mixtures were incubated for an additional 1 h in the absence or presence of 1 μM bisphosphotyrosyl peptide, and PI 3'-kinase kinase activity was measured. The data are expressed as stimulation over the activity of each mixture in the absence of phosphopeptide. The data are the mean ± S.E. from three experiments. D, nSH2-R358A/iSH2/cSH2 (R358A) and nSH2/iSH2-cSH2-R659A (R659A) constructs were incubated with 125I-Bolton-Hunter labeled BPA-bisphosphopeptide at various concentrations of unlabeled peptide for 1 h at 4 °C. The mixtures were then irradiated with UV light (350 nm) for 1 h, boiled in sample buffer, and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Radioactive incorporation was quantitated with a Molecular Dynamics PhosphorImager.
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photyrosyl peptide. PI 3

the nSH2(R358A)-iSH2-cSH2 fragment (Fig. 3

by an additional incubation in the absence or presence of 1 μM bisphosphotyrosyl peptide. PI 3’-kinase kinase activity was measured. The data are expressed as stimulation over the activity of each mixture in the absence of phosphopeptide. The data are the mean ± S.E. from three experiments.

p110α was inhibited to similar extents by the wild-type nSH2-iSH2-cSH2 fragment and the mutant nSH2(R358A)-iSH2-cSH2 and nSH2-iSH2-cSH2(R659A) fragments (Fig. 3B, lanes c-e). This was expected, because others have shown that point mutations within the FLVRES motif do not perturb the overall structure of SH2 domains (18). Phosphopeptides had similar effects on the activity of p110α bound to the nSH2-iSH2-cSH2 and nSH2-iSH2-cSH2(R659A) fragments (Fig. 3C, lanes b and c), with a 100–150% increase in activity. However, phosphopeptides had no effect on the activity of p110α bound to the nSH2(R358A)-iSH2-cSH2 fragment (Fig. 3C, lane d). This was not because of a differences in phosphopeptide binding to the wild type SH2 domain in each construct, because [125I]-Bolton-Hunter labeled phosphotyrosyl peptides bound with similar affinity to the nSH2-iSH2-cSH2(R659A) and nSH2-(R358A)-iSH2-cSH2 fragments (Fig. 3D).

The inability of the cSH2 domain to mediate phosphopeptide activation of the nSH2(R358A)-iSH2-cSH2/p110α dimers was inconsistent with our earlier experiments showing that both the nSH2 and cSH2 domains contributed to phosphotyrosyl peptide activation of p85/p110α dimers (10). The discrepancy could be because of differential post-translational processing of the constructs in bacteria as opposed to insect cells. Alternatively, the cSH2 domain might function differently in the nSH2-iSH2-cSH2 fragment as opposed to intact p85. We therefore produced bacterial GST-fusion proteins containing full-length wild-type p85, as well as p85 containing disabling mutations in the nSH2 and cSH2 domains (R358A and R659A, respectively; Fig. 4A). All of the bacterial p85 constructs inhibited p110α to the same extent as p85 produced in baculovirus-infected SF9 cells (p85SC-9) (data not shown). Incubation of phosphopeptides with either p110α/GST-p85 dimers or p110α/ p85SC-9 dimers increased activity by 120–130% (Fig. 4B, lanes a and b). Phosphopeptides also increased the activity of GST-p85 constructs containing mutant SH2 domains; the activity of nSH2-iSH2-cSH2(R659A)/p110α dimers increased by 90%, and the activity of nSH2(R358A)-iSH2-cSH2/p110α dimers increased by 50% (Fig. 4B, lanes c and d).

These data confirm our earlier report that both N-terminal and C-terminal SH2 can partially mediate phosphopeptide activation of p85/p110α dimers. They also show that the different results obtained with wild-type p85 and the nSH2-iSH2-cSH2 fragment were not because of aberrant processing in bacterial cells. Instead, they suggest that the residues 1–322 of p85 play a role in the regulation of p85/p110α by phosphopeptides. Activation by the cSH2 domain occurred only in the context of intact p85, whereas activation by the nSH2 domain occurred in the absence of the cSH2 domain or residues 1–322 of p85 (the SH3, BCR-homology and proline-rich domains).

DISCUSSION

We have previously shown that p85 is an inhibitor of p110α activity, and that binding of tyrosyl phosphopeptides to the p85 SH2 domains relieves this inhibition (11). This study demonstrates that the SH2 domains of p85 are critical for the inhibitory effects of p85 on p110α. Consistent with previous reports, we find that the iSH2 domain of p85 is sufficient to bind to p110α (14–17). However, iSH2 domain binding alone does not affect p110α activity. Instead, the presence of an SH2 domain linked to the N terminus of the iSH2 domain is required for inhibition of p110α.

Two mechanisms could explain the inhibition of p110α by the nSH2/iSH2 versus iSH2 fragments. The iSH2 domain is predicted to form a coiled-coil domain (14). The presence of an SH2 domain at the N-terminal end of the iSH2 domain could exert a conformational strain on the coiled-coil and alter its interactions with p110α. Alternatively, the nSH2 domain may directly contact p110α, inhibiting its activity. In both mechanisms, conformational changes induced by phosphoprotein binding to the nSH2 domain would relieve the inhibition of...
p110α. We have no direct experimental evidence to distinguish these hypotheses at this time. However, we have noticed that unlike the iSH2 domain itself, a GST-iSH2 fusion protein binds p110α and inhibits its activity by 50%. Inhibition by attachment of a bulky GST moiety to the N terminus of the iSH2 domain is consistent with the first mechanism. Also consistent with this model is a recent paper by Jimenez et al. (1998) describing an oncogenic truncated p85 molecule. Expression of this mutant with p110α caused an increase in activity as compared with wild-type p85, which we would interpret as the loss of p85-induced inhibition of p110α. Since the nSH2 domain is present in the truncation mutant, the loss of inhibition would seem to be because of a conformational change in the iSH2 domain caused by the removal of its extreme C terminus. On the other hand, Cooper and Kashishian reported a direct interaction between p110α and the p85 nSH2 domain in transfected cells (19), which would be consistent with the second mechanism.

Importantly, the iSH2 domain itself neither activates nor inhibits p110α. In contrast, others have suggested that iSH2 domain binding to p110α provides critical activating interactions that are required for p110α activity in mammalian cells (20), and attachment of the iSH2 domain to p110 has been used to produce a constitutively active enzyme (21). However, attachment of bulky moieties such as GST or a tri-HA tag to the N terminus of p110α increases the activity of monomeric p110α activity in mammalian cells by stabilizing the protein (11). Given that the iSH2 domain has no effect on p110α activity in vitro, we think it likely that the iSH2-p110α chimera is active because of the attachment of a bulky group, rather than the provision of specific activating interactions.

A surprising finding in this study is the marked difference in the roles of the nSH2 and cSH2 domains. The nSH2/iSH2 domain fragments bind p110α but have little effect on its activity. Phosphopeptide binding to the cSH2 domain does contribute to p110α activation, but only in the context of the entire p85 protein. Thus, phosphopeptide modulation of p110α via the cSH2 domain appears to be distinct from modulation via the nSH2 domain.

Previous studies have suggested that intramolecular interactions may occur between the SH3 domain and the proline-rich domain of p85 (22). In this case, the cSH2 domain, SH3-PRD domains, and the nSH2 domain may form a compact structure (Fig. 5). Our data would suggest that the nSH2 domain is the major regulator of p110α activity, and that occupancy of the nSH2 domain induces a conformational change (23, 24) that is transmitted to the iSH2 domain and/or p110α (Fig. 5A). In contrast, phosphopeptide occupancy of the cSH2 domain may induce a conformational change that is transmitted to the regulatory nSH2 domain by way of residues 1–322 of p85 (the SH2, Bcr and proline-rich domains) (Fig. 5B). This model would predict that disruption of intramolecular interactions within the SH3 and proline-rich domains of p85 would minimize phosphopeptide-induced activation via the cSH2 domain, but would not affect activation via the nSH2 domain.

Experiments to test this hypothesis are in progress.

In summary, we have shown that the iSH2 domain of p85 mediates binding to p110α, whereas the inhibitory effects of p85 on p110α are largely mediated by an additional constraint imposed by the nSH2 domain. Phosphopeptide occupancy of the nSH2 domain can directly modulate p110α activity. In contrast, modulation of p110α activity by the cSH2 domain occurs by a mechanism that requires residues 1–234 of p85 as well as the nSH2 domain. These studies highlight the complexities of p110α regulation by p85.

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REFERENCES

1. Kapeller, R., and Cantley, L. C. (1994) Bioessays 16, 565–576
2. Otus, M., Hiles, I., Gout, I., Fry, M. J., Ruiz-Larrea, F., Panayotou, G., Thompson, A., Dhand, R., Hsuan, J., Totty, N., Smith, A. D., Morgan, S. J., Courtnedge, S. A., Parker, P. J., and Waterfield, M. D. (1991) Cell 65, 91–104
3. Skolnik, E. Y., Margolis, B., Moghaddam, M., Lowenstein, E., Fischer, R., Deppres, A., Ullrich, A., and Schlessinger, J. (1991) Cell 65, 83–90
4. Escobedo, J. A., Navankassatassias, S., Kavannah, W. M., Miliday, F., Fried, V. A., and Williams, L. T. (1991) Cell 65, 75–82
5. Zheng, Y., Bagrodia, S., and Cerione, R. A. (1994) J. Biol. Chem. 269, 18727–18730
6. Pleiman, C. M., Hertz, W. M., and Cambier, J. C. (1994) Science 263, 1609–1612
7. Rodriguez-Viciana, P., Warner, P. H., Vanhaesebroeck, B., Waterfield, M. D., and Downward, J. (1996) EMBO J. 15, 2442–2451
8. Backer, J. M., Myers, Jr., M. G., Shoelson, S. E., Chin, D. J., Sun, X. J., Miralpeix, M., Hu, P., Margolis, B., Skolnik, E. Y., Schlessinger, J., and White, M. P. (1996) EMBO J. 15, 3469–3479
9. Carpenter, C. L., Auger, K. R., Chamuduri, M., Yoakim, M., Scaffhausen, B., Shoelson, S., and Cantley, L. C. (1993) J. Biol. Chem. 268, 9478–9483
10. Röders-Nikolice, T., Van Horn, D. J., Chen, D., White, M. F., and Backer, J. M. (1995) J. Biol. Chem. 270, 3662–3666
11. Yu, J., Zhang, Y., McIlroy, J., Röders-Nikolice, T., Orr, G. A., and Backer, J. M. (1998) Mol. Cell. Biol. 18, 1379–1387
12. Hiles, I. D., Otus, M., Volinia, S., Fry, M. J., Gout, I., Dhand, R., Panayotou, G., Ruiz-Larrea, F., Thompson, A., Totty, N. F., Hsuan, J. J., Courtnedge, S. A., Parker, P. J., and Waterfield, M. D. (1992) Cell 70, 419–429
13. Escobedo, J., Chen, D. X., Wjazow, C., Michaeli, T., and Backer, J. M. (1997) Mol. Cell. Biol. 17, 244–255
14. Dhand, R., Hara, K., Hiles, I., Bax, B., Gout, I., Panayotou, G., Fry, M. J., Youenazawa, K., Kasuga, M., and Waterfield, M. D. (1994) EMBO J. 13, 511–521
15. Kippel, A., Escobedo, J. A., Hu, Q., and Williams, L. T. (1993) Mol. Cell. Biol. 13, 5560–5566
16. Holt, K. H., Olson, A. L., Maye-Rowley, W. S., and Pessin, J. E. (1994) Mol. Cell. Biol. 14, 42–49
17. Hu, P., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 2577–2583
18. Mayer, B. J., Jackson, P. K., Van Etten, R. A., and Baltimore, D. (1992) Mol. Cell. Biol. 12, 609–618
19. Cooper, J. A., and Kashishian, A. (1993) Mol. Cell. Biol. 13, 1737–1745
20. Jimenez, C., Jones, D. R., Rodriguez-Viciana, P., Gonzalez-Garcia, A., Leonardi, E., Wenzstrom, S., von Kobbe, C., Toran, J. L., R-Borilado, L., Calvo, V., Copin, S. G., Albar, J. P., Gaspar, M. L., Dux, E., Marcos, M. A., R-, Downward, J., Martinez-A., C., Merida, I., and Carrera, A. C. (1998) EMBO J. 17, 743–753
21. Kapeller, A., Escobedo, J. A., Hirano, M., and Williams, L. T. (1994) Mol. Cell. Biol. 14, 2675–2685
22. Hu, Q., Kippel, A., Muslin, A. J., Fantl, W. J., and Williams, L. T. (1995) Science 268, 100–102
23. Kapeller, R., Prasau, K. V. S., Janssen, O., Hwu, W., Schaffhausen, B. S., Rudd, C. E., and Cantley, L. C. (1994) J. Biol. Chem. 269, 1927–1933
24. Shoelson, S. E., Sivaraja, M., Williams, K. P., Hu, P., Schlessinger, J., and Weiss, V. A. (1993) EMBO J. 12, 795–802
25. Panayotou, G., Bax, B., Gout, I., Federwisch, M., Wrobolowski, B., R. Dhand, Fry, M. J., Blundell, T. L., Wallmer, A., and Waterfield, M. D. (1992) EMBO J. 11, 4261–4272