The phosphotyrosine binding (PTB) domain specifically binds to tyrosine-phosphorylated proteins, but differs in structure and mechanism of action from the SH2 domain family. We quantitated the affinity, specificity, and kinetics of the interaction of the SHC PTB domain with a sequence motif, asparagine-X-X-phosphotyrosine (NX(pY)), found in several receptor tyrosine kinases and oncogenic proteins. PTB domain-mediated interaction with the NX(pY) motif of c-ErbB2 was characterized by similar overall affinity but slower kinetics than that reported for SH2 domains. This suggested that unlike SH2 domains, PTB domains may not rapidly exchange among associated proteins. Furthermore, when directly and quantitatively compared, PTB domain binding specificity did not significantly overlap with a panel of seven SH2 domains. Thus, signaling pathways involving PTB and SH2 domain-mediated interactions can be regulated separately. Finally, we define the minimal SHC PTB domain binding motif as NX(pY), not N PX(pY) as suggested by other authors, and suggest a high affinity motif, hydrophobic residue-(D/E)-X-X-X-pY-(W/F), found in the Trk and ErbB receptor tyrosine kinase families. We conclude that PTB domains mediate specific protein-protein interactions independent from those mediated by SH2 domains.

Intracellular signaling by receptor tyrosine kinases is initiated by autophosphorylation of tyrosine residues on the intracellular domains of these molecules (for reviews, see Refs. 1 and 2). One of the most important consequences of autophosphorylation is the creation of binding sites on the receptors for proteins containing domains which specifically interact with tyrosine-phosphorylated targets. The first class of these protein domains to be recognized was the SH2 domain (for reviews, see Refs. 3 and 4). Genetic, biochemical and structural evidence has demonstrated that SH2 domains are functional protein motifs of approximately 100 residues which specifically bind to phosphotyrosine and one or more adjacent amino acids. SH2 domains mediate the assembly of a potentially large number of signaling complexes, within which downstream signaling molecules can be regulated.

Recently, a novel protein domain, the phosphotyrosine binding (PTB) domain, has been described which also specifically binds to the tyrosine-phosphorylated form of target proteins. The PTB domain was first described as a 186-amino acid segment in the NH2 terminus of the signaling adapter protein SHC (5). The SHC PTB domain binds to autophosphorylated growth factor receptors (6–10) and to other unidentified tyrosine-phosphorylated proteins in growth factor-stimulated cells (5). The PTB domain of SHC has functional similarities to the SH2 domain family, but differs in both structure and mechanism of action. Like SH2 domains, PTB domains specifically recognize phosphotyrosine, bind to short sequences, and mediate the assembly of signaling complexes during tyrosine kinase signaling. However, the SHC PTB domain has little sequence homology to the SH2 domain family, has a different predicted secondary structure, and does not contain a functional FLVRES sequence (5), which constitutes part of the phosphotyrosine binding pocket in SH2 domains (11). The SHC PTB domain is likely to be a member of a larger family of similar domains (5, 7, 12).

Unlike SH2 domains, little is known about the binding characteristics of PTB domains. Available data suggest that SH2 domains and PTB domains may recognize different tyrosine-phosphorylated sequences through different mechanisms. Although SH2 domain specificity is principally determined by residues COOH-terminal to the phosphotyrosine (13–17), the PTB domain of SHC requires an asparagine NH2-terminal to the phosphotyrosine, within the motif NXX(pY) (where N is asparagine, X is any amino acid, and pY is phosphotyrosine) (6). Furthermore, peptide ligands bind SH2 domains in an extended conformation; high affinity binding of peptide ligands to the SHC PTB domain may require a specific conformation conferred by multiple residues in the peptide (6). From these studies, several receptor tyrosine kinases have been predicted to contain binding sites for the SHC PTB domain. The affinity, specificity, and kinetics of PTB domain-mediated interactions have not been quantitatively determined. These data are critical to understanding the relationship of PTB and SH2 domain-mediated interactions during tyrosine kinase signaling. In this report, we examined quantitatively the binding of the SHC PTB domain to sequence motifs derived from growth factor receptors and oncogenes. This data strongly suggest that PTB domains mediate specific protein-protein interactions during tyrosine kinase signaling.

**MATERIALS AND METHODS**

Expression of GST Fusion Proteins—The NH2-terminal and COOH-terminal SH2 domains of the 85-kDa subunit of phosphatidylinositol 3'-kinase (p85) were expressed in Escherichia coli as glutathione S-transferase (GST) fusion proteins tagged with the influenza hemaglutinin (HA). The phosphotyrosine motif of c-ErbB2 was characterised by similar overall affinity but slower kinetics than that reported for SH2 domains. This suggested that unlike SH2 domains, PTB domains may not rapidly exchange among associated proteins. Furthermore, when directly and quantitatively compared, PTB domain binding specificity did not significantly overlap with a panel of seven SH2 domains. Thus, signaling pathways involving PTB and SH2 domain-mediated interactions can be regulated separately. Finally, we define the minimal SHC PTB domain binding motif as NX(pY), not N PX(pY) as suggested by other authors, and suggest a high affinity motif, hydrophobic residue-(D/E)-X-X-X-pY-(W/F), found in the Trk and ErbB receptor tyrosine kinase families. We conclude that PTB domains mediate specific protein-protein interactions independent from those mediated by SH2 domains.

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tin epitope (HA), as described previously (18). The SHC2 domain of SHC was expressed from baculovirus as a HA-tagged GST fusion protein as described (5). The NH2- and COOH-terminal SH2 domains of phosphatase C-γ (PLC-γ) and GTPase Activating Protein (GAP) were expressed as TrpE fusion proteins as described (19). All proteins were harvested by lysis in lysis buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 0.15 unit/ml aprotinin, and 20 μM leupeptin) and clarified by centrifugation at 13,000 × g.

The PTB domain of SHC (residues 46-232) was expressed as a glutathione S-transferase fusion protein from baculovirus as described (5, 20). For real-time kinetic analysis, the PTB domain sequence was tagged with the glutathione S-transferase (GST) epitope (21) at the NH2 terminus, down to the baculovirus expression vector pAC13 (22), and expressed as described (5). The Glu-Glu-tagged PTB domain was purified by affinity chromatography with a monoclonal anti-Glu-Glu antibody (23), followed by chromatography with HiTrap Q anion exchange resin (Pharmacia LKB Biotech). The purity of this preparation was >95% as assessed by SDS-polyacrylamide gel electrophoresis and silver staining.

Peptides—Phosphotyrosine-containing peptides were synthesized and HPLC purified by Chiron Mimotopes Peptide Systems, Melbourne, Australia. Purity by HPLC and by mass spectroscopy was >70%; in most cases purity was much higher than 70%, and contaminants were mostly small peptides. As indicated, some peptides were biotinylated by the NH2 terminus. Peptides were dissolved in 50 mM NaPO4 buffer, pH 6.5, and stored under nitrogen at −80°C. The peptides synthesized are listed in Table 1. b-P85 sequence was derived from the mouse platelet-derived growth factor β receptor (PDGF) residues 709−730; b-GAP from PDGF residues 729−749, and b-PLC from PDGFR residues 857−876. Peptides—Phosphotyrosine-containing peptides were synthesized and HPLC purified by Chiron Mimotopes Peptide Systems, Melbourne, Australia. Purity by HPLC and by mass spectroscopy was >70%; in most cases purity was much higher than 70%, and contaminants were mostly small peptides. As indicated, some peptides were biotinylated by the NH2 terminus. Peptides were dissolved in 50 mM NaPO4 buffer, pH 6.5, and stored under nitrogen at −80°C. The peptides synthesized are listed in Table 1. b-P85 sequence was derived from the mouse platelet-derived growth factor β receptor (PDGF) residues 709−730; b-GAP from PDGF residues 729−749, and b-PLC from PDGFR residues 857−876.

ELISA Binding Assays—1 μg of antibody 12CA5, a monoclonal anti-βI antibody directed against the HA epitope (Boehringer Mannheim), was adsorbed to each well of microtiter plates (ImmunoMax). Excess antibody was then removed, and the plates blocked in ELISA buffer (20 mM HEPES, pH 7.7, 7.5% w/v KCl, 0.1 mM EDTA, 2.5 mM MgCl2, 0.05% Triton X-100, and 0.1% bovine serum albumin (containing) 3% bovine serum albumin). The plates were washed and then incubated with Sf9 cell lysates containing HA-tagged GST-PTB domain fusion protein for 2 h at 4°C. Alternatively, bacterial cell lysates containing HA-tagged GST SH2 domain fusion proteins were similarly added to the plates. The amount of fusion proteins were normalized to be the same in each well as judged by SDS-polyacrylamide gel electrophoresis and Western blotting with 12CA5 antibody. As controls, 12CA5 antibody and/or fusion protein was omitted from some wells. The plates were then washed three times, and incubated with dilutions of biotinylated, phosphotyrosine-containing peptides diluted in ELISA buffer for 1−2 h at 4°C. For competition assays, 0.25−125 μM biotinylated peptide b-NL(pY)(pY) was added with the indicated concentrations of nonbiotinylated, tyrosine-phosphorylated competing peptides. The plates were washed again and incubated with streptavidin-coupled alkaline phosphatase (1 unit/ml, Boehringer Mannheim) in ELISA buffer for 10 min at room temperature. The plates were washed twice more and incubated with developing buffer (100 mM glycine, pH 10.1, 1 mM ZnCl2, 1 mM MgCl2, and 1 mg/ml p-nitrophenol phosphate) for 5−10 min at room temperature. Linear kinetics for the color development reaction under these conditions were confirmed by real-time spectrophotometric measurements. Color development was stopped by the addition of 0.1 M EDTA and quantitated by OD at 405 nm. Unless otherwise stated, all results are reported as the average of duplicates, ± range, and have had background (binding in the absence of PTB domain) subtracted. Where representative experiments are shown, data are typical of at least three experiments.

Immunoprecipitation Binding Assays—Equal amounts of TrpE-SH2 domain protein were mixed with the indicated concentrations of biotinylated, tyrosine-phosphorylated peptides in ELISA buffer, immunoprecipitated with anti-TrpE (Oncogene Science) antibodies, washed, and assayed for the presence of bound peptide with streptavidin-coupled alkaline phosphatase, essentially as described previously (6) and above.

Table 1

| Name of peptide | Amino acid sequence |
|-----------------|---------------------|
| b-NL(pY)(pY)    | Biotin-PAFSPAPDNL(pY)(pY) WQDNNSEGG |
| b-p85           | Biotin-OMDSDK5ED5I(pY) VPMNLMKGDIK |
| b-PLC           | Biotin-AYGQGSGNED(pY) I1PLPDPDPKPD |
| b-GAP           | Biotin-IKAYKDEIS5(pY) MAPYDNVPS |
| erbB2           | AFDFNLNY(WDQNS |
| erbB3           | AFDNPOYWHSR |
| Trk             | I1ENPQYIFSDA |
| MT              | LLSNTP-push |
| EGFR            | SLDNPOYQQDF |
| IR              | ASSNPY(pY)LSAS |

3.4 mM EDTA, and 0.005% Tween. To capture the peptide on the chip surface, 0.9 nmol of biotinylated NL(pY)(pY) peptide was mixed with a 7-fold molar excess of biotin in a final volume of 120 μl of HEPES/P20 buffer and injected over the chip surface at a flow rate of 5 μl/min at 25°C. Highly purified Glu-Glu-tagged PTB domain protein was diluted to the indicated concentrations in HEPES/P20 buffer and injected at 5 μl/min over 7 min. Chips were washed with 3.5 ml of NaSCN, pH 5.85.

Data processing was with BLAevaluation software (Pharmacia, version 2.1). Dissociation rate constants were measured in buffer flow according to the following equation,

\[
R_1 = R_0 e^{-t/(R_0 e^{R_0 t})} \quad (Eq. 1)
\]

where \( R_0 \) and \( R_1 \) are the relative response at the starting time and at \( t \), respectively. \( R_0 \) is the steady state response level, \( R_1 \) is the association rate constant, and \( C \) is the molar concentration of PTB domain. Dissociation constants \( K_d \) were calculated by dividing \( R_0 \) by \( R_1 \).

RESULTS

Affinity and Kinetic Measurements of SHC PTB Domain Binding—Using a solution binding assay, we previously reported that a biotinylated tyrosine-phosphorylated peptide derived from the c-ErbB2 intracellular domain, containing the sequence Asn-Leu-phosphotyrosine-phosphotyrosine-b-NL(pY)(pY) (Table 1), binds to the SHC PTB domain, as do other peptides containing the NXX(pY)pY motif (6). This peptide has the highest apparent affinity for the SHC PTB domain of the sequences tested to date. To further characterize this interaction, we performed additional measurements of the kinetics of PTB domain binding to this peptide using biospecific interaction analysis (BlAcore (24, 25)). In this technique, peptide ligands are immobilized on a gold-coated surface and exposed to solutions of PTB domain protein in a flow chamber. The amount of protein which associates with the immobilized peptide is quantitated by measurement of a surface plasmon resonance signal, from which parameters of binding can be calculated.

The results of a typical experiment are shown in Fig. 1. PTB domain protein bound to immobilized peptide b-NL(pY)(pY) (Fig. 1), but did not bind significantly to uncoated or streptavidin-coated chips in the absence of phosphorylated peptide, nor to immobilized, nonphosphorylated peptide containing the same amino acid sequence (not shown). These data confirm our earlier report that PTB domain binding requires the presence of phosphotyrosine. The rate of dissociation of PTB domain from the immobilized b-NL(pY)(pY) peptide under conditions of buffer flow was linear, with a rate constant \( k_d \) of 2.5 ± 0.21 s−1. The calculated association rate constant \( k_a \) was 4.7
A typical resonance signal, which reflects PTB domain binding to the peptide, is given in arbitrary resonance units (RU). A typical experiment is shown.

Specificity of PTB Domain-mediated Interactions—We first reported that the SHC PTB domain recognizes an asparagine residue NH₂-terminal to the phosphotyrosine, while SH2 domains recognize principally COOH-terminal residues. Subsequent reports have confirmed those findings (10, 27). These results predict that SH2 and PTB domains may have different specificities and bind to different phosphorylated targets, but the extent to which their specificities actually overlap has not been examined quantitatively. To test specificity directly, we measured the binding of the SHC PTB domain to several peptides recognized by SH2 domains, and conversely, measured binding of a panel of SH2 domains to a high affinity peptide ligand for SHC PTB domain.

We have developed an ELISA assay in a microtiter plate format for measuring binding of the SHC PTB domain to peptide ligands (see "Materials and Methods"). 12CA5, a monoclonal antibody directed against the influenza HA epitope, was adsorbed to microtiter plates and used to capture HA-tagged GST-PTB domain fusion protein. Biotinylated peptide ligands were then incubated with the immobilized PTB domain, the plates washed, and the presence of bound peptide detected by addition of streptavidin-coupled alkaline phosphatase and a colorimetric phosphatase substrate. No color development above background was observed in the absence of 12CA5 antibody or PTB domain (Fig. 2A). Saturable binding was observed with increasing concentrations of biotinylated peptide derived from ErbB2 (b-NL(pY)(pY)), with half-maximal binding (EC₅₀) at approximately 20–50 nM in multiple experiments (Fig. 2A).

This value is consistent with the Kᵢ derived from real-time kinetic analysis of approximately 53 nM (above) and with competition binding experiments performed in solution (6). No binding was observed with biotinylated, nonphosphorylated peptides (not shown). Therefore, the ELISA binding assay is a valid method for quantitating PTB domain-mediated interactions.

Although the b-NL(pY)(pY) peptide bound with high affinity to the SHC PTB domain, biotinylated peptides containing recognition sequences for SH2 domains from the 85-kDa subunit of phosphatidylinositol 3'-kinase (p85), from GAP (b-GAP), or from PLC-γ (b-PLC) did not bind with measurable affinity to the SHC PTB domain in the same experiment (Fig. 2B). These same peptides did bind to their respective SH2 domains in separate experiments (Fig. 3 and data not shown). Therefore, under these conditions, the PTB domain does not recognize these SH2 binding sites. None of these peptides contains an Asn at position −3 relative to the phosphotyrosine, although peptide b-PLC has an Asn at the −2 position. These data are consistent with our previous report that an Asn at the −3 position is necessary for PTB domain binding and show that an Asn at the −2 position is not sufficient for binding.

To examine the affinity of SH2 domains for the PTB domain recognition sequence, we performed ELISA binding assays of HA-tagged SH2 domains with the PTB peptide ligand b-NL(pY)(pY). The COOH- and NH₂-terminal SH2 domains of p85 bound to peptide b-NL(pY)(pY) with a half-maximal binding concentration at least 2 orders of magnitude greater than that of PTB domain for the same peptide (Fig. 3A). However, a peptide containing the recognition sequence for the p85 SH2 domain (b-p85, Table I) bound to p85 SH2 domains with a half-maximal concentration of 40 nM in the same experiment (data not shown). This affinity is similar to that of PTB domain for peptide b-NL(pY)(pY) (Fig. 2). Similarly, the affinity of the SHC SH2 domain was at least 1000 times lower for peptide b-NL(pY)(pY) than the affinity of PTB domain for this peptide (Fig. 3B).

To examine the binding of other SH2 domains to this peptide, we used a solution binding assay described previously (6). TrpE-SH2 domain fusion protein and biotinylated peptides were mixed in solution, immunoprecipitated with anti-TrpE antibodies, washed, and bound peptide detected as described above. No binding of the b-NL(pY)(pY) peptide was detected to either the NH₂-terminal or the COOH-terminal SH2 domains of PLC-γ, even at relatively high concentrations of peptide (Fig. 3C).

A peptide containing a PLC-γ recognition motif bound to the NH₂-terminal SH2 domain of PLC-γ in the same experiment (Fig. 3C). Similar results were obtained with the NH₂-terminal and COOH-terminal SH2 domains of GAP (Fig. 3D).
Binding of the SHC PTB Domain to NXX(pY) Motifs from Growth Factor Receptors—The binding sites for the full-length SHC molecule on several growth factor receptors and oncogenic proteins include NXX(pY) motifs (28–32) that are predicted to be potential SHC PTB domain binding sites (6–8, 10, 27). However, this possibility has not been tested quantitatively.

We measured the relative affinity of the SHC PTB domain for tyrosine-phosphorylated peptides representing the SHC binding sites from these proteins. Competition binding experiments were performed in the ELISA format, in which a fixed concentration of biotinylated peptide b-NL(pY)(pY) (closed circles). Controls included assays performed in the absence of the capturing antibody, 12CA5 (open squares), or in the absence of PTB domain protein (open triangles). A representative experiment is shown. B, binding of PTB domain to peptides representing binding sites for the SH2 domains of GAP (open squares), PLC-γ (open triangles), and p85 (open circles), compared with peptide b-NL(pY)(pY) (closed circles).
that SH2 domain-containing proteins can rapidly exchange among associated proteins. Rapid dissociation would also allow for rapid control of SH2 domain-mediated interactions, by continually exposing SH2 domain binding sites to phosphorylation and dephosphorylation by regulatory enzymes. Our data suggest that PTB domains do not rapidly exchange as do SH2 domains and therefore may be regulated differently. Furthermore, the differences in binding kinetics support the idea that PTB domain-mediated interactions have a different structural basis for phosphotyrosine recognition than the SH2 domains.

Second, our results demonstrate directly that the affinity of a panel of seven SH2 domains for the b-NL(pY)pY) peptide was at least several orders of magnitude lower than the affinity of PTB domain for this peptide. Furthermore, the PTB domain does not recognize several SH2 domain target sequences. Therefore the specificities of these SH2 domains and the SHC PTB domain are significantly different. This suggests that in vivo PTB and SH2 domains mediate separate protein-protein interactions and therefore may be regulated independently. Consistent with this concept, the SH2 and PTB domains of SHC bind different proteins in Balb/c 3T3 cells (5). PTB and SH2 domains may also cooperate in signal complex assembly by binding to separate sites on the same molecule.

Finally, we have identified a candidate high affinity binding motif for the SHC PTB domain by quantitatively comparing PTB binding to predicted target sequences derived from various signaling proteins. This motif, ψ(D/E)-N-X-X-pY-(W/F), is present in the Trk tyrosine kinase receptor family, including Trk A (nerve growth factor receptor), Trk B (BDNF receptor), and Trk C (NT-3 receptor); the ErbB family, including ErbB2, ErbB3, and ErbB4; in torso, a receptor tyrosine kinase from Drosophila, and in dozens of other proteins. These data are consistent with our previous report of the effects of single amino acid substitutions of ErbB2-derived peptides on binding to the SHC PTB domain (6). In those studies, the presence of an aspartic acid at the −4 position (relative to the phosphotyrosine) and either a tryptophan or phenylalanine at the +1 position conferred higher affinity.

These results do not exclude the possibility that the peptides with lower affinities, such as those derived from the EGF and insulin receptors, may represent PTB domain binding sites in vivo. The competitive binding assay was used to compare the relative affinities of these SHC binding sites for the PTB domain, but the true affinities are critically dependent on assay conditions and may be underestimated in Fig. 4. Furthermore, these sequences may confer higher affinity in the context of the intact protein. We have previously suggested that the conformation of the PTB domain binding site is important for high-affinity interaction (6). Recent reports have demonstrated that the SHC PTB domain can interact with the insulin receptor (7) and with EGF receptor in vitro (8, 9); the affinities of these interactions were not determined.

It has been proposed by other authors that the SHC PTB domain consensus binding site is the motif asparagine-proline-X-phosphotyrosine (NPX(pY)) (7–10, 27). The data in this report confirm our earlier conclusion (6) that NPX(pY) is not the only motif recognized by the SHC PTB domain. The proline at the −2 position is not necessary for high affinity binding (Figs. 1, 2, and 4) (6, 10). In quantitative assays, the only two residues which are absolutely required for binding are the asparagine at the −3 position and the phosphotyrosine (6). In fact, when compared directly, most NPX(pY)-containing sequences bind with lower affinity than does the ErbB2-derived NLY(pY) motif (Fig. 4) (6). Therefore, current data suggest that it is more appropriate to define the minimal SHC PTB domain binding motif as NXX(pY). The proline in the −2 position may serve to
enhance affinity in some contexts, as do multiple other residues.

In conclusion, this report addresses several of the fundamental questions raised by the identification of PTB domains. First, we show directly that the specificity of the SHC PTB domain is different from that of a panel of SH2 domains and is likely to be different from most SH2 domains. Second, the SHC PTB domain binds to targets with similar affinity but different kinetics from SH2 domains. Finally, we have defined a high-affinity binding site for the SHC PTB domain which is likely to be different from that of a panel of SH2 domains and is likely to be similar from most SH2 domains. Weshow directly that the specificity of the SHC PTB domain is enhanced in some contexts, as do multiple other residues.

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REFERENCES

1. Kavanaugh, W. M., and Williams, L. T. (1995) in Modular Texts in Molecular Cell Biology (Heldin, C.-H., and Purton, M., eds) Chapman & Hall Ltd., London, in press
2. van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) Annu. Rev. Cell Biol. 10, 251–337
3. Cohen, G. B., Ren, R., and Baltimore, D. (1995) Cell 80, 237–248
4. Pawson, T. (1995) Nature 373, 573–580
5. Kavanaugh, W. M., and Williams, L. T. (1994) Science 266, 1862–1865
6. Kavanaugh, W. M., Turk, C. W., and Williams, L. T. (1995) Science 268, 1177–1179
7. Gustafson, T. A., He, W., Craparo, A., Schaub, C. D., and O’Neill, T. J. (1995) Mol. Cell. Biol. 15, 2500–2508
8. van der Geer, P., Willey, S., Ka-Man Lai, V., Olivier, J. P., Gish, G. D., Stephens, R., Kaplan, D., Shoelson, S., and Pawson, T. (1995) Curr. Biol. 5, 404–412
9. Blakie, P., Immanuel, D., Wu, J., Li, N., Yajnik, V., and Margolis, B. (1994) J. Biol. Chem. 269, 32031–32034
10. Dikic, I., Batzer, A. G., Blakie, P., Obermeier, A., Ullrich, A., Schlessinger, J., and Margolis, B. (1995) J. Biol. Chem. 270, 15125–15129
11. Mayer, B. J., Jackson, P. K., Van Etten, R. A., and Baltimore, D. (1992) Mol. Cell. Biol. 12, 609–618
12. Bork, P., and Margolis, B. (1995) Cell 80, 693–694
13. Songyang, Z., Shoelson, S. E., McGlade, J., Olivier, P., Pawson, T., Bustelo, X. R., Barbadid, M., Sabe, H., Hanafusa, H., Schlessinger, J., Baltimore, D., Ratnoffsky, A., Feldman, R. A., and Cantley, L. C. (1994) Mol. Cell. Biol. 14, 2777–2785
14. Zhou, S., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Hasser, W. G., King, F., Roberts, T., Ratnoffsky, S., Lechleider, R. J., Neel, B. G., Birge, R. B., Fajardo, J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B., and Cantley, L. C. (1993) Cell 72, 767–776
15. Escobedo, J. A., Kaplan, D. R., Kavanaugh, W. M., Turk, C. W., and Williams, L. T. (1991) Mol. Cell. Biol. 11, 1125–1132
16. Cantley, L. C., Auger, K. R., Carpenter, C., Dudowsk, B., Graziani, A., Kaper, R., and Saltos, S. (1991) Cell 64, 281–302
17. Bibbins, K. B., He, W., Craparo, A., Schaub, C. D., and O’Neill, T. J. (1995) J. Biol. Chem. 270, 32031–32034
18. Klippel, A., Escobedo, J. A., Hirano, M., and Williams, L. T. (1994) Mol. Cell. Biol. 14, 2675–2685
19. Fantl, W. J., Escobedo, J. A., Martin, G. A., Turk, C. W., del Rosario, M., McCormick, F., and Williams, L. T. (1992) Cell 69, 413–423
20. Summers, M. D., and Smith, G. E. (1987) A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Texas Agricultural Station, College Station, TX
21. Rubinfeld, B., Munemitsu, S., Clark, R., Conroy, L., Watt, K., Crosier, W. J., McCormick, F., and Polakis, P. (1995) Mol. Cell. Biol. 15, 5977–5982
22. Grussmeyer, T., Scheidtmann, K. H., Hutchinson, M. A., Eckhart, W., and Walter, G. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 7952–7954
23. Felder, S., Zhou, M., Hu, P., Urena, J., Ulrich, A., Chaudhuri, M., White, M., Shatkin, J. A., and Schlessinger, J. E., and McCormick, S. E., and Schlessinger, J. (1995) Mol. Cell. Biol. 15, 1449–1455
24. Panayotou, G., Gish, G., End, P., Triuong, O., Gout, I., Dhanda, R., Fray, M. J., Hiles, I., Pawson, T., and Waterfield, M. D. (1993) Mol. Cell. Biol. 13, 3567–3576
25. (1995) BI A evaluation 2.1 Software Handbook, Pharmacia Biosensor AB, Uppsala, Sweden
26. Songyang, Z., Margolis, B., Chaudhuri, M., Shoelson, S. E., and Cantley, L. C. (1994) J. Biol. Chem. 270, 14863–14866
27. Obermeier, A., Lammers, R., Weismuller, K. H., Jung, G., Schlessinger, J., and Ullrich, A. (1993) J. Biol. Chem. 268, 22963–22966
28. Oshayash, Y., Kido, Y., Okutani, T., Sugimoto, Y., Sakaguchi, K., and Kasuga, M. (1994) J. Biol. Chem. 269, 18674–18678
29. Segatto, O., Pelicci, G., Giuli, S., Digiesi, G., Di Fiore, P. P., McGlade, J., Pawson, T., and Pelici, P. G. (1993) Oncogene 8, 2105–2112
30. Campbell, K. S., Ogris, E., Burke, B., Su, W., Auger, K. R., Druker, B. J., Schaffhausen, B. S., Roberts, T. M., and Pallas, D. C. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 6344–6348
31. Prigent, S. A., and Gullick, W. J. (1994) EMBO J. 13, 2831–2841
Affinity, Specificity, and Kinetics of the Interaction of the SHC Phosphotyrosine Binding Domain with Asparagine- X-X-Phosphotyrosine Motifs of Growth Factor Receptors

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