Binding Specificity and Mutational Analysis of the Phosphotyrosine Binding Domain of the Brain-specific Adaptor Protein ShcC*

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Shc proteins (hereafter referred to as ShcA) represent major substrates of tyrosine phosphorylation by a wide variety of growth factors and cytokines. We have recently described a novel ShcA-like protein, ShcC, which like ShcA contains an NH2-terminal phosphotyrosine binding domain (PTB), a central effector region (CH1) and a COOH-terminal Src homology 2 domain (SH2). Both the SH2 and PTB domains of ShcC bind a similar profile of proteins as the comparable regions of ShcA. In an effort to define the functional differences or similarities between ShcA and ShcC, we have further characterized the PTB domain of ShcC. Using a degenerate phosphopeptide library screen, we show that the PTB domain of ShcC preferentially binds the sequence His-hydrophobic-Asn/hydrophobic-Asn-Pro-Ser/Thr-Tyr(P). This sequence is similar to the binding site for the SchA PTB domain, suggesting that these two proteins may have overlapping specificities. In addition, random mutagenesis of the ShcC PTB domain has identified several amino acids important for PTB function (Gly32, Glu63, Ala136, Gly139, and Asp140). Mutation of these amino acids dramatically reduces the affinity of the ShcC PTB domain for the activated epidermal growth factor receptor in vitro.

Tyrosine phosphorylation represents a critical switch in the regulation of cell growth, differentiation, and development. Phosphorylation of cellular proteins on tyrosine residues creates high affinity binding sites for proteins containing Src homology 2 (SH2) domains. SH2 domains recognize tyrosine and the 3–6 amino acids COOH-terminal to the phosphotyrosine. The selectivity of a particular SH2 domain is dictated by these COOH-terminal amino acids. Recently another phosphotyrosine binding domain (PTB) has been described (1–3). This domain, also known as PH (phosphotyrosine interaction domain) and SAIN (Shc and IRS-1 NPXY binding), recognizes phosphotyrosine in the context of amino acids NH2-terminal to the phosphotyrosine. Thus, PTB and SH2 domains represent distinct protein modules that recognize tyrosine-phosphorylated proteins, but under entirely different contexts.

PTB domains were first described in the adaptor protein ShcA (1, 2). ShcA represents a major target of tyrosine phosphorylation following stimulation by a variety of growth factors and cytokines (4). Upon activation of receptor tyrosine kinases, ShcA becomes physically associated with the receptor and phosphorylated on tyrosine. This association was initially believed to occur through the SH2 domain of ShcA (5). Indeed, the ShcA SH2 binding site on the EGF-R was mapped using a combination of in vitro binding and phosphopeptide competition assays (6, 7). The peptide selectivity of the ShcA SH2 domain was determined using a degenerate phosphopeptide library screen (8). These results suggested that a number of receptors had putative ShcA binding sites. However, some confusion arose as to the true ShcA binding site due to the finding that ShcA association with the polyoma virus middle T antigen occurred through an Asn-Pro-Thr-Tyr sequence and not the consensus ShcA SH2 binding sequence (9, 10). In addition, the association of ShcA with a 145-kDa phosphoprotein in platelet-derived growth factor-stimulated cells was shown to occur not through the SH2 domain, but rather through the NH2-terminus (2). The determination that ShcA contains two distinct phosphotyrosine binding motifs, a COOH-terminal SH2 and a NH2-terminal PTB, provided an explanation for these observations. PTB recognition sites are also present in the nerve growth factor receptor (TrkA), the insulin and insulin-related receptors, interleukin-2 receptor, and the EGF-R.

We have recently described the identification of two shc-like genes which we called shcB and shcC (11). shcB is nearly identical in sequence to the partial human shc-like gene sck and most likely represents the mouse homolog of this gene (11). shcC, however, has not yet been found in other organisms. In contrast to the wide expression of shcA, shcC is restricted in expression to tissues of neural origin, suggesting a role for this adaptor protein in brain-specific tyrosine kinase signaling. Like ShcA, ShcC contains an NH2-terminal PTB domain, a central proline-rich region (CH1) and a COOH-terminal SH2 domain. In addition, ShcC binds to activated growth factor receptors through both its SH2 and PTB domains. In this report, we have further characterized the PTB domain of ShcC. We have determined the phosphopeptide selectivity of the ShcC PTB domain as well as describe a number of point mutations that dramatically reduce the affinity of the ShcC PTB domain for activated growth factor receptors. These mutations occur in conserved regions of the PTB domain, suggesting an important role for these amino acids in phosphotyrosine recognition and binding.

**EXPERIMENTAL PROCEDURES**

Peptide Library Screen—The peptide library used for these studies has the sequence Met-Ala-X-X-Asn-X-Tyr(P)-X-Ala-Lys-Lys-Lys, where X corresponds to any amino acid except for Trp and Cys. This library was synthesized as described previously (8). The theoretical
temperature as described previously (11). Approximately equal
were prepared from A431 cells stimulated with EGF for 2 min at room
Shelton Earp and used at a concentration of 100 ng/ml. Briefly, lysates
degeneracy of this library is 18 or approximately 3.4 × 10^7. Determina-
tion of the binding specificity was done as described previously (12).

Random Mutagenesis and Bacterial Expression—The PTB domain of
ShcC (amino acids 28–212) was amplified by polymerase chain reaction
(PCR) as described previously (11) and then either directly subcloned
into the pGEX bacterial expression plasmids or randomly mutagenized
with hydroxylamine treatment (13, 14), subcloned into pCR1 (Invitro-
gen) for sequence analysis, and then subcloned into pGEX. For hydroxyl-
amine mutagenesis, 30 μl of a PCR fragment encoding the PTB
domain was mixed with 150 μl of hydroxylamine solution
(0.5 M hydroxylamine, 0.2 M sodium pyrophosphate, pH 6.0) and then
heated to 70°C for 5 min. To this mixture was added 16 μl of hydroxylamine solution
(0.5 M hydroxylamine, 0.2 M sodium pyrophosphate, pH 6.0) and then
heated to 70°C for 20 min. After this incubation 80 μl of stop solution
(0.6 M Tris, pH 8.0, 1.0 M NaCl, 20% acetic acid) was added and the mutagenized DNA was purified over a G-50-Sepharose column equilibrated
with TE (10 mM Tris, pH 7.5, 1 mM EDTA). The resulting DNA was subcloned into the pCR1 vector using the TA Cloning Kit (Invitrogen).

Point mutations were identified by dye-deoxy sequence analysis. All the
PTB fragments were subcloned into pGEX vectors as BamHI-EcoRI frag-
ments. Mutants 4a and 4b were constructed by digesting the pGEX-
PTB constructs for the wild-type and mutant 4 PTB domains with
BstEII, which produces two fragments of approximately 2 kbp (encoding
amino acids 28–59 of the PTB) and 3.4 kbp (encoding the amino acids
60–213 of the PTB domain). Mutant 4a was constructed by ligating the 3.4-kbp wild-type fragment with the 2-kbp fragment from mutant 4.
Mutant 4b was constructed by ligating the 3.4-kbp fragment from mutant 4 with the 2-kbp wild-type fragment. Plasmid constructs were sequenced to confirm the presence of each mutation. Mutant proteins were expressed in E. coli strain DH5α at 37°C and purified on
glutathione-agarose beads after induction with IPTG for 3 h. Beads containing GST-PTB were washed with phosphate-buffered saline containing
20% glycerol, 0.5% Tween 20, 1 mM diithiothreitol, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, resuspended in a 50% slurry, and then stored at −70°C. Aliquots of each PTB domain were fractionated on
SDS-polyacrylamide gel electrophoresis and the gel stained with
Coomassie to assess concentrations.

In Vitro Binding Assays—A431 cells (a human epidermoid carci-
noma) were grown in Dulbecco’s modified Eagle’s medium containing
10% fetal bovine serum, penicillin, and streptomycin in the presence of
5% CO₂. Epidermal growth factor (EGF) was a kind gift of Dr. H. Shelton Earp and used at a concentration of 100 ng/ml. Briefly, lysates
were prepared from A431 cells stimulated with EGF for 2 min at room
temperature as described previously (11). Approximately equal

A number of groups have defined the sequence requirements of a phosphopeptide for binding the ShcA PTB domain (12, 15–18) (Table I). The ShcA and ShcC PTB domains share a high degree of amino acid sequence homology (78% identity; Fig. 1). Given our interest in further defining functional differences or similarities between ShcA and ShcC, we have examined the peptide specificity of bacterially expressed ShcC PTB using a degenerate phosphopeptide library screen (see “Experimental Procedures”). Using this strategy, the ShcA PTB was shown to select phosphopeptides containing the sequence Asn-Pro-X-Tyr(P)-Phe-X-Arg with the strongest selectivity at posi-

RESULTS AND DISCUSSION

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![Fig. 1. Alignment of PTB domains.](image1)

![Fig. 2. Phosphopeptide selectivity of the PTB domain of ShcC.](image2)
Phosphopeptide selectivity at the Asn-Pro-Ser/Thr-Tyr(P) (Fig. 2 and Table I). There also appears to be selectivity for small chain amino acids (Table I). Phosphorylation site of TrkA (11). In addition, both ShcC and ShcA PTB domains bind a 170-kDa phosphoprotein in EGF-stimulated A431 cell lysates (Fig. 3).

The numbers in parenthesis indicate enrichment values as described in Refs. 8 and 33. Only those amino acids with enrichment values greater than 1.4 are shown. Amino acids with enrichment values greater than two are indicated in boldface.

**Table I**

| ShcC PTB | ShcA PTB | ShcC SH2 | ShcA SH2 |
|----------|----------|----------|----------|
| Tyro(M) | 6 | Y (2.9) | N (2.8) | P (2.7) | S (2.7) | Tyr(P) | F (2.1) |
| F (1.9) | F (2.4) | F (1.7) | F (2.4) | M (1.2) | F (2.1) | G (1.8) |
| Y (1.8) | I (2.0) | I (1.6) | I (1.6) | Y (1.7) |
| L (1.7) | H (1.6) | L (1.5) | Y (1.6) |

a The numbers were taken from Ref. 11. For determining selectivity, a library was used in which the amino acids were degenerate at positions 2 and 3.

b The amino acid at the Tyr(P) position was fixed as Asp for determining the ShcC PTB selectivity as described under "Experimental Procedures."

c These numbers were taken from Ref. 12. For determining selectivity, a library was used in which the amino acids were degenerate at positions 2 and 3.

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ShcC PTB Binding Selectivity and Mutagenesis

Fig. 3. Mutations in the PTB domain of ShcC altered the binding to the activated EGFR. A, in vitro binding of the ShcC PTB domain to the activated EGFR. GST fusion proteins for each PTB were purified and used in an in vitro binding experiment, as described previously (11). Western blots were probed with anti-phosphotyrosine and anti-GST antibodies. Signals were quantitated on a Bio-Rad phosphorimager. B, quantitation of PTB binding data shown in A. Shown are the binding affinities for the wild-type and mutant 4 PTB domains relative to wild-type. These results are the average of three independent binding assays with different preparations of GST-PTB protein. The graph on the right represents the results of binding experiments with the wild-type PTB, mutant 4 PTB, and the mutant 4 PTB derivatives 4a and 4b. Standard errors are indicated with bars.

| Summary of mutations in the ShcC PTB domain | PTB mutant | Mutation | Location of mutation | Relative binding |
|---------------------------------------------|------------|----------|----------------------|------------------|
| Wild type                                  |            |          |                      | 1.0              |
| 1                                           | T118S      | Loop 5   | (β2–β3)              | 0.81 ± 0.13      |
| 2                                           | E63G       | α2       |                      | 0.087 ± 0.037    |
| 3                                           | M130I      | Loop 7   | (β4–β5)              | 1.0 ± 0.31       |
| 4                                           | S132V      | Loop 7   | (β4–β5)              | 0.11 ± 0.048     |
| 4a                                          | G32R       | Loop 1   | (α1–β1)              | 0.79 ± 0.11      |
| 4b                                          | A136T      | β5       |                      | 0.52 ± 0.11      |
| 4b                                          | C166R      | Loop 10  | (β7–α3)              | 1.0 ± 0.12       |
| 5                                           | T58A       | α2       |                      | 1.2 ± 0.20       |
| 6                                           | G30R       | α1       |                      | 1.2 ± 0.20       |
| 7                                           | H128Q      | Loop 7   | (β4–β5)              | 0.073 ± 0.0067   |
| 8                                           | G139E      | Loop 8   | (β5–β6)              | 1.2 ± 0.35       |
| 9                                           | D140N      | Loop 8   | (β5–β6)              | 1.2 ± 0.10       |
| 10                                          | V36M       | β1       |                      | 1.0 ± 0.23       |

*The nomenclature used corresponds to that used in Ref. 21.

The relative binding affinities for mutants 4a and 4b were determined in independent binding experiments with the wild-type and mutant 4 PTB domains only. In these experiments, the relative binding affinities for the wild-type and mutant 4 PTB domains were 1 and 0.055 ± 0.019, respectively. Quantitation of the results from these experiments is shown in Fig. 3B.

During the course of this study, the NMR solution structure of the PTB domain of ShcC complexed to a TrkA phosphopeptide was described (21). The tertiary structure of the PTB domain is composed of two antiparallel β-sheets formed by a series of seven β strands and three α helices. The overall topology of the PTB domain bears a striking resemblance to that of another modular domain, the pleckstrin homology (PH) domain, although these two domains lack any sequence homology. The ShcA and ShcC PTB domains share a high degree of sequence identity. Overall these two domains are 78% identical particularly in the regions that form specific contacts with the phosphopeptide as determined by NMR (21). For example, the β5 strand is 100% identical in ShcA and ShcC and forms four contacts with the phosphopeptide ligand (21). These findings suggest that the solution structure of the ShcC PTB domain may be very similar to that of ShcA. Therefore, we have analyzed our mutations using the ShcA PTB structure as a framework for comparison. The E63G mutation occurs in the middle of α2 helix, which connects the β1 and β2 strands. These β strands comprise part of a β-sheet that forms a hydrophobic pocket into which the phosphopeptide binds. Thus, the E63G mutation likely disrupts important ionic interactions with the α2 helix, thereby abrogating phosphopeptide recognition. The G139E/D140N mutations occur in a loop between the β5 and β6 strands. The β5 strand forms several contacts with the phosphopeptide backbone (21). Gly139 appears important for forming a proper turn between these two β strands, which allows for their antiparallel arrangement. Thus, the G139E/D140N double mutant likely disrupts these contacts by restricting the ability of the loop to form a turn, thereby disrupting the alignment of β5 and β6 and diminishing the affinity of the PTB for phosphopeptide.

The three mutations present in PTB mutant 4 occur in different regions of the PTB domain. Of particular interest is the fact that the A136T mutation occurs in the β5 strand, which forms part of the sheet into which the phosphotyrosine binds (21). Several amino acids in this β strand, including Ala136, are in close proximity with the phosphopeptide. Mutation of Ala136 likely disrupts these contacts, thereby abrogating binding to the activated EGFR. Thus, the A136T mutation likely accounts for the majority of the reduction in EGFR binding by mutant 4. The A136T/C166R double mutant does not appear to be as impaired in binding as the triple mutant (Fig. 3C). Although we have not assessed the individual effects of these two substitutions, we believe that the C166R mutation may have some compensatory effect in the context of the double mutant. This compensation in binding may not occur in the context of the triple mutant.

In addition to mutations which affect phosphotyrosine binding, a number of PTB mutants are unaffected in their interaction with the activated EGFR (Fig. 3). Many of these mutations represent conservative substitutions that likely do not have a profound affect on the structure or interactions with other amino acids within the PTB domain itself or the phosphopeptide. Many of the nonconservative substitutions occur in loop regions that tend to be more resistant to mutational effects due to the ability of these regions to move freely in space.

We have identified several mutations in the PTB domain of a novel adaptor protein, ShcC, which dramatically reduce the affinity of its PTB domain for the activated EGFR. Based on the predicted structure of the ShcA PTB domain, these mutations likely disrupt regions important in phosphopeptide recognition and binding. Several groups have identified additional mutants in the ShcA PTB domain that affect PTB binding to phospho-
tyrosine containing proteins (18, 20, 21). Interestingly, Yajnik et al. (20) independently isolated an Ala to Thr mutation at amino acid 153 in ShcA, which is identical to the A136T mutation present in mutant 4 of ShcC. This mutation resulted in a 74% reduction in binding of the ShcA PTB to the activated EGFR, further supporting the notion that the A136T mutation of mutant 4 is indeed the critical amino acid mutation affecting binding.

Mutation of Arg175 of ShcA to either Glu, Met, or Lys completely abolishes phosphotyrosine binding, suggesting a critical role for this Arg in substrate binding (18, 21). Based on the recently described structure of the ShcA PTB domain, Arg175 directly participates in binding the phosphotyrosine residue of the phosphopeptide ligand. Interestingly, this Arg is not absolutely conserved in all PTB domains (19). This finding is in contrast to SH2 domains, which contain an absolutely conserved Arg, mutation of which blocks SH2 binding to tyrosine-phosphorylated proteins (22, 23). In addition to Arg175, mutation of Phe198 drastically reduces binding (~1% of wild type) (20). Phe198, in contrast to Arg175, is conserved in the majority of PTB domains described thus far (19, 20). Interestingly, the IRS-1 PTB domain binds to a similar sequence as the ShcA and ShcC PTB domains, yet shares no apparent sequence homology. These observations suggest that although different PTB domains may lack primary sequence homology, they may adopt similar three-dimensional structures. Indeed, the PTB domain of ShcA and the PH domain of pleckstrin share a similar three-dimensional structure in the absence of sequence homology (21). Alternatively, PTB domains lacking a comparable Arg175 as found in the PTB domains of Shc family members may adopt a different structure and, thus, employ a different mechanism for phosphopeptide recognition and binding. Determining the structures of other PTB domains will address these possibilities.

We have examined the peptide selectivity of the ShcC PTB domain. This PTB domain has a similar selectivity as compared with the PTB domain of ShcA. Similar results were obtained with the SH2 domains of Shc family members (11). The similarity in the sequence and the peptide selectivities of both ShcA and ShcC PTB and SH2 domains suggests that these two adapter proteins may share overlapping functions, but in different cell types. Indeed, both proteins interact with similar receptors and tyrosine-phosphorylated proteins in vitro (11). The identification of mutant PTB domains presents the possibility of designing ShcC dominant interfering mutants that may block signaling from tyrosine kinases as well as other proteins that signal through Shc family members.

In addition to binding tyrosine-phosphorylated proteins, the PTB domain of ShcA has also been shown to bind phospholipids (21). Furthermore, the SH2 domains of phosphatidylinositol 3’-kinase, as well as Src and Abl, have been shown to bind phospholipids (24). This interaction with lipids provides a possible explanation for how ShcA may translocate to the membrane to activate Ras in the absence of direct binding to activated growth factor receptors (25-28). In addition, the interaction of ShcA with phospholipids may play a role in the regulation of phospholipid metabolism.

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REFERENCES

1. Blaikie, P., Immanuel, D., Wu, J., Li, N., Yajnik, V., and Margolis, B. (1994) J. Biol. Chem. 269, 32031–32034
2. Kavamura, W. M., and Williams, L. T. (1994) Science 266, 1862–1865
3. Gustafson, T. A., He, W., Craparo, A., Schaub, C. D., and O'Neill, T. J. (1995) Mol. Cell. Biol. 15, 2500–2508
4. Pawson, T. (1995) Nature 373, 573–579
5. Pelicci, G., Lanfrancone, L., Grignani, F., McGlade, J., Cavallo, F., Forni, G., Nicotelli, I., Grignani, F., Pawson, T., and Pelicci, P. G. (1992) Cell 70, 93–104
6. Batzer, A. G., Rotin, D., Urena, J. M., Skolnick, E. Y., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 5192–5201
7. Okabayashi, Y., Kids, Y., Okutani, T., Sugimoto, Y., Sakaguchi, K., and Kasuga, M. (1994) J. Biol. Chem. 269, 18674–18678
8. Songyang, Z., Shoest, S. E., McGlade, J., Olivier, P., Pawson, T., Bustelo, X. R., Barbadid, M., Sanchez, H., Hanafusa, H., Yi, T., Ren, R., Baltimore, D., Ratnofsky, A., Felman, R. A., and Cantley, L. C. (1994) Mol. Cell. Biol. 14, 2777–2785
9. 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
10. Dilworth, S. M., Brewer, C. E., Jones, M. D., Lanfrancone, L., Pelicci, G., and Pelicci, P. G. (1994) Nature 367, 87–90
11. O'Bryan, J. P., Songyang, Z., Cantley, L., Der, C., and Pawson, T. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 2729–2734
12. Songyang, Z., Margolis, B., Chaudhuri, M., Shoest, S., and Cantley, L. C. (1996) J. Biol. Chem. 270, 14863–14866
13. Feig, L. A., Corbely, M., Pan, B.-T., Roberts, T. M., and Cooper, G. M. (1987) Mol. Endocrinol. 1, 127–136
14. Quilliam, L. A., Kato, K., Rabun, K., Hisaka, M., Huff, S., Campbell-Burk, S., and Der, C. J. (1994) Mol. Cell. Biol. 14, 1113–1121
15. Kavanaugh, W. M., Turk, C. W., and Williams, L. T. (1995) Science 266, 1177–1179
16. Batzer, A. G., Blaikie, P., Nelson, K., Schlessinger, J., and Margolis, B. (1995) Mol. Cell. Biol. 15, 4403–4409
17. Wolf, G., Trub, T., Ottinger, E., Groninga, L., Lynch, A., White, M., Miyazaki, M., Lee, J., and Shoest, S. (1995) J. Biol. Chem. 270, 27407–27410
18. van der Geer, P., Wiley, S., Gish, G., Lai, V. K.-M., Stephens, R., White, M., Kaplan, D., and Pawson, T. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 963–968
19. Bork, P., and Margolis, B. (1995) Cell 80, 693–694
20. Yajnik, V., Blaikie, P., Bork, P., and Margolis, B. (1996) J. Biol. Chem. 271, 1813–1816
21. Zhou, M.-M., Meadows, R. P., Logan, T. M., Yoon, H. S., Wade, W. S., Ravichandran, K. S., Burakoff, S. J., and Feik, S. W. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 7784–7788
22. Marengere, L. E., and Pawson, T. (1992) J. Biol. Chem. 267, 22779–22786
23. Watanabe, G., Shodden, S. E., Pant, N., Cowburn, D., and Kuriyan, J. (1993) Cell 72, 779–790
24. Rameh, L. E., Chen, C.-S., and Cantley, L. C. (1995) Cell 83, 821–830
25. Li, N., Schlessinger, J., and Margolis, B. (1994) Oncogene 9, 3457–3465
26. Gotto, N., Tojo, A., Muraya, K., Hashimoto, Y., Hattori, S., Nakamura, S., Takenawa, T., Yazaki, Y., and Shibuya, M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 167–171
27. Soer, C. A., Alvarez, C. V., Beguinot, L., and Carpenter, G. (1994) Oncogene 9, 2207–2215
28. Fredell, Y.-W. C., Jin, Y., Quilliam, L. A., Burchert, A., McCloskey, P., Spizz, G., Varnum, B., Der, C., and Liu, E. T. (1996) Mol. Cell. Biol. 16, 135–145
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