A Novel Phosphotyrosine Motif with a Critical Amino Acid at Position −2 for the SH2 Domain-mediated Activation of the Tyrosine Phosphatase SHP-1*

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SHP-1 is a protein-tyrosine phosphatase associated with inhibition of activation pathways in hematopoietic cells. The catalytic activity of SHP-1 is regulated by its two SH2 (Src homology 2) domains; phosphotyrosine peptides that bind to the SH2 domains activate SHP-1. The consensus sequence (I/V)XYY(I/V) is present in the cytoplasmic tails of several lymphocyte receptors that interact with the second SH2 domain of SHP-1. In several of these receptors, there are two or three occurrences of the motif. Here we show that the conserved hydrophobic amino acid preceding the phosphotyrosine is critical for binding to and activation of SHP-1 by peptides corresponding to sequences from killer cell inhibitory receptors. The interaction of most SH2 domains with phosphopeptides requires only the phosphotyrosine and the three residues downstream of the tyrosine. In contrast, the shortest peptide able to bind or activate SHP-1 also included the two residues upstream of the phosphotyrosine. A biphosphopeptide corresponding to the cytoplasmic tail of a killer cell inhibitory receptor with the potential to interact simultaneously with both SH2 domains of SHP-1 was the most potent activator of SHP-1. The hydrophobic residue upstream of the tyrosine was also critical in the context of the biphosphopeptide. The contribution of a hydrophobic amino acid two residues upstream of the tyrosine in the SHP-1-binding motif may be an important feature that distinguishes inhibitory receptors from those that provide activation signals.

Receptor-mediated activation of cellular responses is often initiated by the activation of tyrosine kinases. The signal is propagated by the sequential recruitment of proteins to the phosphorylated targets of the kinases. SH2 (Src homology 2) domains are protein modules that specifically bind to phosphotyrosine residues and are found in a variety of proteins such as protein kinases and adapter molecules. A general feature of all SH2 domains is a conserved pocket that binds the phosphotyrosine and often incorporates a hydrophobic residue at the third position (reviewed in Ref. 1). This specificity is provided by specific pockets that bind the phosphotyrosine and +3 residues as revealed by structural studies of several SH2 domains complexed with phosphopeptides (1).

A small family of protein-tyrosine phosphatases contain SH2 domains. This family is composed of mammalian SHP-1 and SHP-2 and the Drosophila homologue of SHP-2, corkscrew. These protein-tyrosine phosphatases are important regulators of many cellular signaling processes (reviewed in Ref. 2). SHP-2 is broadly expressed and is important for activation signals through several different growth factor receptors. In contrast, SHP-1 is expressed predominantly in hematopoietic cells and has been implicated in inhibition of signaling through growth factor, cytokine, and antigen receptors. These protein-tyrosine phosphatases contain two SH2 domains in tandem and a single catalytic domain. The protein-tyrosine phosphatase activity is negatively regulated by the SH2 domains in that their removal or occupancy by phosphopeptides increases the phosphatase activity of the catalytic domain (3–8). The SH2 domains of SHP-1 and SHP-2 are more closely related to each other than to any other SH2 domains known, with their next closest relatives being the SH2 domains of phosphatidylinositol 3-kinase and phospholipase C-γ1 (9). It is of particular interest to understand the specificity of these SH2 domains because they control both the localization and activation of these phosphatases.

The optimal binding sequences for SH2 domains have been defined with degenerate phosphopeptide libraries and pooled sequence analysis. The NH₂-terminal SH2 domain (SH2N domain) of SHP-2 binds preferentially to pY(I/V)(V/I)(V/I) (9), and the SH2N domain of SHP-1 binds preferentially to pYXF(X)F (10). The predictive value of these motifs was supported by the presence of appropriate sequences in several receptors that bind to the SH2N domain of SHP-1, such as c-Kit, and erythropoietin and interleukin-3 receptors. Due to a strong sequence similarity to the SH2N domains of both SHP-1 and SHP-2, the second SH2 domain (SH2C domain) of SHP-1 is predicted to have a similar preference for hydrophobic residues at positions +1 and +3 (10).

In contrast to the binding studies that considered only residues downstream of the phosphotyrosine, we identified the motif (I/V)XYYXXL by sequence alignment of inhibitory receptors known to recruit SHP-1 (11). These include the B lymphocyte receptors CD22 (12) and FcγRIIB (13) and the family of killer cell inhibitory receptors (KIR) expressed in natural killer and T lymphocytes (11). Peptides derived from KIR and FcγRIIB have been reported to bind to the SH2C domain of SHP-1 (11, 19), whereas intact CD22 has been reported to

*The abbreviations used are: SH2N domain, NH₂-terminal SH2 domain; SH2C domain, COOH-terminal SH2 domain; FcγRIIB, Fcγ receptor type IIb; KIR, killer cell inhibitory receptor(s); GST, glutathione S-transferase; p-NPP, p-nitrophenyl phosphate.

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interact with the SH2N domain (14). The motif is also found in NKG2A (11, 15), a molecule associated with a receptor complex recently shown to be involved in inhibition of human natural killer cells (16), and in gp49, a molecule with inhibitory potential expressed in mouse mast cells (17) and in natural killer cells (18). A related motif, VXXXY, was found in Ly-49 molecules (11), another family of inhibitory receptors that are expressed in mouse natural killer cells (19). Phosphopeptides derived from Ly-49 interact with the SH2 domains of both SHP-1 and SHP-2 (20). The inhibitory receptors expressed in natural killer cells, including human KIR and NKG2, as well as the mouse inhibitory receptors Ly-49 and gp49 also share the sequence QEVT just upstream of the tyrosine.

Several recent observations have raised questions about the specificity of the interaction of receptors with the (L/V)XXX(L/V) motif and SHP-1. SHP-1 was implicated in the inhibitory signals of FcγRIIB because of a defect in the receptor’s signaling in B cells from SHP-1-deficient mice (13). Recently, it has been reported that FcγRIIB functions in mast cells independently of SHP-1 and associates with the inositol phosphatase SHIP, which also contains an SH2 domain (21). Catalytically inactive SHP-1 acts as a dominant-interfering molecule for the inhibitory signals delivered by KIR (11), and SHP-1 associates with the receptor upon tyrosine phosphorylation (11, 20, 22, 23). However, it remained possible that the dominant-interfering mutant of SHP-1 exerted its effect by preventing the association of a different protein with tyrosine-phosphorylated KIR, which was itself important in providing the inhibitory signal. In addition, studies with metabolically labeled cells show the association of KIR with a protein that corresponded in size to SHP-1 as well as several unidentified proteins (23). To address this issue, we have analyzed proteins that bind to synthetic phosphotyrosine peptides corresponding to cytoplasmic tail sequences of KIR and FcγRIIB.

To determine whether the amino acids identified in the consensus sequence (L/V)XXX(L/V) are important for interaction with SHP-1, we have examined the specificity of the interaction of sequences containing this motif with SHP-1 and the contribution of these residues to the activation and binding of SHP-1. The data established a critical role for the amino acid two residues upstream of the tyrosine in SHP-1 binding and activation.

EXPERIMENTAL PROCEDURES

Peptides—All peptides were purchased from Quality Controlled Biochemicals, Inc. (Hopkinton, MA) and supplied at a purity of >98%. The phosphotyrosyl residue was incorporated during peptide synthesis. The peptides were synthesized by Fmoc (N-(9-fluorenylethoxycarbonyl) chemistry, purified by reversed-phase high pressure liquid chromatography, and analyzed for purity by ion spray mass spectrometry and 1H NMR. Phosphopeptides were dissolved at 0.057 mM and coupled to Affi-Gel-10 beads as instructed by the manufacturer (Bio-Rad) at a ratio of 1 ml of peptide to 1 ml of settled beads. The sequence of peptide Y1Y2 is EQDPQEVTVAYQLNSVFTQKTRPSQRPKTPTDIVYTELPNA. pY1Y2, Y1pY2, and pY1pY2 have the same sequence as Y1Y2 with the first, second, or both tyrosines phosphorylated, respectively. The sequence of pY1pY2-Y2A is EQDPQEAAT(pY)AYQLNSVFTQKTRPSQRPKTPTDIVYPTELPNA.

Silver Stain—Cell lysates were prepared from bulk human T cell populations cultured in the presence of 100 units/ml recombinant interleukin-2 (gift of Hoffmann-La Roche). The cells were lysed at 34°C for 10 min in a buffer (1% Triton X-100, 0.15 M NaCl, and 0.057 mM peptide). 11.4 nmol of peptide were incubated in the mixture was incubated at 4°C for 90 min. Samples were washed three times in lysis buffer and analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting with anti-SHP-1 antibodies or with anti-GST antibodies (Upstate Biotechnology, Inc., Lake Placid, NY) using the chemiluminescence detection system (Pierce).

RESULTS

Binding of SHP-1 to Phosphotyrosine Peptides Derived from KIR—To assess the relative specificity of the cytoplasmic tail sequences of KIR and FcγRIIB for SHP-1, proteins that associate with phosphopeptides coupled to beads were analyzed by silver staining after separation by SDS-polyacrylamide gel electrophoresis. The phosphopeptide derived from the FcγRIIB sequence bound two major proteins from T cell lysates, corresponding in size to SHP-1 and FcγRIIB, respectively (Fig. 1). In contrast, phosphopeptides derived from the first tyrosine in the KIR cytoplasmic tail or corresponding to the biphasophorylated KIR sequence each bound to a single major protein that corresponded in size to SHP-1. Western blotting confirmed the presence of SHIP at 155 kDa in the FcγRIIB peptide-associated proteins and of SHP-1 at 62 kDa in both the FcγRIIB and KIR peptide-associated proteins (data not shown). A control tyrosine-phosphorylated peptide corresponding to a sequence in the T cell receptor ζ-chain did not detectably bind to proteins in cell lysates (Fig. 1). By this method, the tyrosine-phosphorylated sequence from Ly-49 did not appear to bind to any protein. Similar data were obtained with lysate prepared from human natural killer cells or from a human B cell line (data not shown). In experiments using mouse cell lines as a source of lysate, weak bands were detected for Ly-49 at 62 and 66 kDa (data not shown). Therefore, whereas all sequences with the motif (L/V)XXX(L/V) interact with SHP-1, only FcγRIIB was

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able to interact with both SHP-1 and SHIP.

**Activation of SHP-1 by Phosphopeptides Derived from Lymphocyte Receptors That Bind SHP-1**—To directly test the role of residues at upstream of phosphotyrosine in inhibitory motifs. Proteins were isolated from total cell lysates of T lymphocytes by phosphotyrosine-containing peptides coupled to beads and analyzed by SDS-polyacrylamide gel electrophoresis and silver staining of the gel. The peptides are indicated at the top of the lanes, and their sequences are listed in Table I. The apparent molecular masses of marker proteins are indicated (in kDa) on the right. The bands with apparent molecular masses corresponding to SHP-1 (62 kDa) and SHIP (155 kDa) are indicated by the *arrows* on the left.

**Residues Upstream of Phosphotyrosine Are Important for Activation of SHP-1**—To directly test the role of residues at positions 1 and 2 in SHP-1 activation, a set of KIR-pY1 analogues were synthesized with substitutions at these positions (Table II). Substitution of threonine at position –1 with histidine (found in CD22-pY5) or leucine (found in CD22-pY5) did not substantially affect SHP-1 activity (Fig. 2C). However, an alanine in this position partially compromised the peptide’s activity. The effect of substitutions at position –2 provided clear evidence for the importance of this residue in SHP-1 activation (Fig. 2B). Substitution of valine with isoleucine, the residue found at this position in the CD22-pY5, FcγRIIB, and KIR-pY2 peptides, increased the activation of SHP-1. A conservative substitution with leucine did not significantly alter the activity of the peptide. All other substitutions tested (alanine, serine, aspartate, and arginine) seriously compromised the ability of the peptide to activate SHP-1 (Fig. 2D).

To control for the possibility that position –2 had an influence on dephosphorylation of the peptide by SHP-1, we tested the various peptides in an assay that compared them as substrates of the isolated phosphatase domain of SHP-1 (Fig. 3). Although differences in the ability of SHP-1 to dephosphorylate the various peptides were obvious, there was no correlation between resistance to dephosphorylation by GST-catSHP-1 and the ability to activate full-length SHP-1. For example, pY1-2A, which failed to activate SHP-1, is not a better substrate than KIR-pY1. Also of interest, pYAQL was a worse substrate than KIR-pY1 (data not shown). Therefore, the influence of position –2 is most likely at the level of interaction with the SH2 domains of SHP-1.

The hierarchy in the ability of peptides from different receptors to activate SHP-1 and the conservation of the sequence GLpYQGL in the receptors expressed in natural killer cells suggested that positions other than position –2 could be important for achieving optimal activation of SHP-1. Therefore, another series of peptides with various substitutions in the KIR-pY1 sequence were generated (Table II) and tested for their ability to activate SHP-1. Substitutions with alanine at position –4 caused a partial reduction of SHP-1 activity (data not shown), but not below levels obtained with the KIR-pY2 or Ly-49 peptide. Individual substitution at position –6 and double substitutions at positions –4 and –3 did not modify the ability of the peptide to activate SHP-1 (data not shown).

**Role of Residues Downstream of Phosphotyrosine in Activation of SHP-1**—The reduced activation of SHP-1 by Ly-49 as compared with that by KIR-pY1 can be explained by their difference at position +3. Substitution of leucine at position +3 in KIR-pY1 with valine (found in Ly-49) diminished the activation of SHP-1 to the same level as that obtained with the Ly-49 peptide (Table III). These data indicate that the SHP-1 SH2 domains prefer leucine over valine at position +3. Two chimeric peptides, initially designed to test whether residues upstream of the YXXL motif were sufficient to dictate recognition by SHP-1, revealed that residues +1 and +2 and perhaps residues +4 and +5 may also contribute to the interaction with SHP-1. The chimeric peptides pY1/+ and pY1/Y2 (Table II) behaved similarly to pY5 and KIR-pY2, respectively (Table III). Therefore, residues at positions +1, +2, +4, or +5 could interfere with the interaction of SHP-1 with the motif YXXYL.

**Binding of Phosphopeptides to SHP-1 Correlates with Their Ability to Activate SHP-1**—The relationship between activation of SHP-1 and peptide binding to SHP-1 was examined. The majority of the peptides listed in Table II were also tested for their ability to compete with the binding of SHP-1 to KIR-pY1 coupled to beads. Representative experiments are shown in Fig. 4. The nonactivating peptide p5-pY5 did not compete with KIR-pY1, whereas soluble KIR-pY1 competed efficiently (Fig. 4A, top and middle panels). Similar to the weaker activation of SHP-1 by KIR-pY2 relative to that by KIR-pY1, the ability of KIR-pY2 to compete with KIR-pY1 for binding to SHP-1 was weak.
below that of KIR-pY1 (Fig. 4A, middle panel). The ability of KIR-pY1 peptides with substitutions at position −2 to compete for binding also correlated with their ability to activate SHP-1. These results reiterated the importance of position −2 in the consensus motif (I/V)LXYXX(L/V). The effect of position −2 was also observed for interaction with the isolated SH2C domain of SHP-1 (Fig. 4B).

The only peptide for which the binding and activation data did not correlate perfectly was CD22-pY5. CD22-pY5 was the most potent monophosphopeptide for activation of SHP-1, whereas its efficiency in the competitive binding assay was equivalent to that of KIR-pY1. Given that KIR-pY1 binding was detectable with the isolated SH2C domain and not with the SH2N domain (11), the competition assay likely measures the relative affinity of peptides for the SH2C domain. However, CD22 has been reported to interact with the SH2N domain (14). An interaction of CD22-pY5 with both SH2C and SH2N domains of SHP-1 could explain why it is a better activator of SHP-1 because activation of SHP-1 by phosphopeptides has been reported to be more efficient through the SH2N domain (8, 27). On the other hand, the relative resistance of the CD22 peptide to the phosphatase activity of SHP-1 (see Fig. 3) may explain its higher performance in the protein-tyrosine phosphatase assay with p-NPP as a substrate.

Interaction of the KIR-derived peptides and the isolated SH2N domain of SHP-1 was not readily detectable (11). However, biphosphopeptides spanning both tyrosines in the KIR sequence bind SHP-1 with a greater affinity than the corre-
sponding monophosphopeptides (22), suggesting that these bi-
phosphopeptides may interact simultaneously with the SH2C
and SH2N domains. The ability of such long biphosphopeptides
to activate SHP-1 in vitro as compared with the mixture of the
individual phosphopeptides was measured (Fig. 5). The left-
ward shift of the curve demonstrated that the biphosphopep-
tide pY1pY2 is the best activator of SHP-1. The much lower
level of activity of the peptide pY1pY2-2A confirmed that posi-
tion 2 is also important in the context of the biphosphopep-
tide. The activity of pY1Y2 and Y1pY2 was similar to that of
the corresponding 14-amino amino peptides pY1 and pY2, re-
spectively. Therefore, the high activity of pY1pY2 is due to the
phosphate moiety on both tyrosines and not to the intervening
sequence.

**DISCUSSION**

Phosphopeptides corresponding to KIR-pY1 or KIR-pY1pY2
bound a single major protein in lymphocyte lysates that comi-
grated with SHP-1. These observations support the conclusion
that a dominant-interfering form of SHP-1 prevents KIR func-
tion by specifically competing with SHP-1 (11, 28). In contrast,
a phosphopeptide corresponding to the FcγRIIB cytoplasmic
tail bound two major proteins, one of which comigrated with
SHP-1 and one with the inositol phosphatase SHIP. These
results support the reports of FcγRIIB-mediated inhibition by
either SHP-1 or SHIP (13, 21). More important, these results
indicate that the (I/V)X[YXX] consensus motif contains ele-
ments that are important for binding and activation of SHP-1,
but not SHIP.

Short peptides corresponding to the KIR-pY1 sequence
showed that the minimal consensus sequence (I/V)X[YXX] was
sufficient to confer most of the activity of the KIR-pY1 peptide.
The short peptide VTpYAQL activated SHP-1, whereas pYAQL

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**Table II**

| Peptide | Amino acid sequence |
|---------|---------------------|
| KIR-pY1 | E Q D P Q E V T pY A Q L N H |
| pYAQL  | Ac- – – – – – – – – – – – – – |
| VTpYAQL| Ac- – – – – – – – – – – – – – |
| EVTPYAQL| Ac- – – – – – – – – – – – – – |
| QEVTPYAQL| Ac- – – – – – – – – – – – – – |
| pY1-4A | – – – – A – – – – – – – – |
| pY1-4A | – – – – A – – – – – – – – |
| pY1-6A | – – A – – – – – – – – – – |
| pY1-6A | – – A – – – – – – – – – – |
| pY1-2I | Ac- – – – – – – – – – – – – |
| pY1-2I | Ac- – – – – – – – – – – – – |
| pY1-2L | Ac- – – – – – – – – – – – – |
| pY1-2L | Ac- – – – – – – – – – – – – |
| pY1-2R | Ac- – – – – – – – – – – – – |
| pY1-2R | Ac- – – – – – – – – – – – – |
| pY1-1A | – – – – – – – – – – – – – |
| pY1-1A | – – – – – – – – – – – – – |
| pY1-1H | – – – – – – – – – – – – – |
| pY1-1H | – – – – – – – – – – – – – |
| pY1-1L | – – – – – – – – – – – – – |
| pY1-1L | – – – – – – – – – – – – – |
| pY1+3V | Ac- – – – – – – – – – – – – |
| pY1+3V | Ac- – – – – – – – – – – – – |
| pY1pY2 | – – – – – – – – – – – – – |
| pY1pY2 | – – – – – – – – – – – – – |
| pY1/ζ | – – – – – – – – – – – – – |
| pY1/ζ | – – – – – – – – – – – – – |

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**Table III**

| Peptide | Relative activity |
|---------|------------------|
| KIR-pY1 | 3.1 2.9 2.3 2.8 |
| KIR-pY2 | 1.6 1.6 1.4 1.4 |
| Ly-49-pY1 | 1.8 1.7 |
| pY1pY2 | 1.7 1.9 |
| pY1+3V | 1.2 1.4 |
| pY1pY2 | 1.0 1.2 |
| pY1+3V | 0.9 0.9 1.1 |

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* a Dashes indicate identity to KIR-pY1. Ac indicates acetylation and NH₂ indicates amidation at the amino and carboxyl termini of the peptide, respectively.
A Phosphopeptide Motif for Activation of SHP-1

Competition by soluble phosphopeptides for SHP-1 binding to KIR-pY1. A, SHP-1 was isolated from a Jurkat cell lysate by KIR-pY1 coupled to beads in the absence (first two lanes) or presence of the indicated soluble peptides. The triangles indicate titration of soluble peptide from left to right corresponding to 140, 47, 15.5, and 5.2. The amount of SHP-1 associated with the KIR-pY1 beads was detected by Western blot analysis. Each panel spans the region of the gel between the 46- and 97-kDa markers. SHP-1 migrated at 66 kDa. Protein degradation during preparation of the lysate is likely to account for the double band observed in some of the Jurkat cell lysates. B, SHP-1 localization and SHP-1 detection was by Western blotting with anti-GST antibody; and the peptide titration corresponds to 140, 47, 15.5, 5.2, and 1.7. The portion of the gel shown is between 35 and 50 kDa.

The importance of residue −2 was clear in the context of the highly active biphosphopeptide pY1pY2. This result is particularly relevant because a long peptide is more likely than a short one to adopt the conformation of the actual protein. In fact, the peptide tested included 45 of the 76 amino acids of the KIR cytoplasmic tail. Although short peptides rarely exhibit a fixed conformation in aqueous environments, it is possible that the upstream residues invoke a conformation favorable for the interaction of the peptide and the SH2 domain. However, such an explanation is unlikely because pYAQL was not able to activate SHP-1.

The crystal structure of the SH2 domains of the related protein-tyrosine phosphatase SHP-2 complexed with phosphopeptides suggest how position −2 may be involved in peptide binding to the SH2 domains of SHP-1. These structures revealed that the peptide-binding groove accommodates residues −2 to +5 relative to the phosphotyrosine (30, 31). In contrast to a peptide complexed with the SH2 domain of Src (32), residue −2 is in contact with the SH2 domains of SHP-2 (30, 31). Based on their sequence similarity to SHP-2, the SH2 domains of SHP-1 are likely to share the feature of an extended peptide-binding groove relative to prototypic SH2 domains. The peptide from the platelet-derived growth factor receptor crystallized with SHP-2 has a valine at position −2. In the structure, the side chain of the valine caps the phosphotyrosine-binding pocket. Although not all the peptides that bind to SHP-2 contain a similar residue at position −2, the valine at position −5 in the platelet-derived growth factor receptor peptide has been shown to be important for binding and activation of SHP-2 (33). It is possible that the interaction with the tyrosine ring and the side chain of residue −2 provides an entropic contribution for binding. Such an interaction may be required in general for the binding of peptides to the SH2C domain of SHP-1. The phosphotyrosine position in the SH2 domains of SHP-2 is different than in typical SH2 domains (30) and may contribute to the need for the extra interactions. Alternatively, a hydrophobic pocket in the SH2C domain of SHP-1, but not present in SHP-2, could explain how Ile/Val/Leu at position −2 contributes to the binding of peptides to the SHP-1 SH2C domain. Perhaps these interactions compensate for a lack of interaction with position +1 and become necessary for the sequences from the lymphocyte receptors that lack a hydrophobic residue at position +1. Of note, there are interactions reported for KIR- and Ly-49-derived peptides with SHP-2, but these interactions are quite weak (20).

A long groove for peptide binding can impose stringent re-
requirements for many of the residues downstream of a phosphotyrosine. In the structural studies of SHP-2, interactions of both crystallized peptides with the protein occurred for side chains +3 and +5 (30). Such a long groove may exclude the majority of peptides due to steric interference. In support of this notion, the upstream portion of pY1 did not confer activity to the downstream portions of KIR-pY2 and pY5 in terms of SHP-1 activation. This suggests that pY5 and KIR-pY2 could lack other necessary residues downstream of the tyrosine. The lack of consensus at any of these positions in the sequences that lack other necessary residues downstream of the tyrosine. In the structural studies of SHP-2, interactions of requirements for many of the residues downstream of a phosphopeptide to activate SHP-1. This motif differs from the usual binding motifs of SH2 domains by the inclusion of tifs of lymphocyte-activating receptors. This motif differs from the sequences in the immune receptor tyrosine-based activation motif of residue substitution with alanine at position +3 have also been implicated for high affinity binding of phosphopeptides to the related SH2N domain of SHP-2 (34).

The issue of which SH2 domain is involved with recognition of residue −2 is intriguing. A specific interaction of the peptides with the SH2N domain alone was not observed. Thus, it is tempting to presume that the SH2C domain is the only relevant domain. However, the ability of the biphosphopeptide to produce such strong activation as compared with the mono-phosphopeptides suggests that both tyrosines and both SH2 domains are involved in the interaction. The interaction with the SH2N domain may be of low affinity and therefore is not detected in isolation. However, when the two phosphotyrosines are linked, a high avidity interaction is generated. An even more dramatic example of enhanced activation by biphosphopeptides has been observed for SHP-2 (7). More important, substitution with alanine at position −2 drastically decreased the ability of the biphosphopeptide to activate SHP-1.

We have provided evidence that the consensus residues (I/V/L/YXXX(L/V)) are critical amino acids for binding and activation of SHP-1. Ile/Val/Leu at position −2 differentiates the YXXL sequences of inhibitory receptors from the YXXL sequences in the immune receptor tyrosine-based activation motifs of lymphocyte-activating receptors. This motif differs from the usual binding motifs of SH2 domains by the inclusion of upstream residues.

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