Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 1141–1145, February 1996
Biochemistry

Differential functions of the two Src homology 2 domains in protein tyrosine phosphatase SH-PTP1

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ABSTRACT **SH-PTP1 (also known as PTP1C, HCP, and SHP) is a non-transmembrane protein tyrosine phosphatase (PTPase) containing two tandem Src homology 2 (SH2) domains. We show here that the two SH2 (N-SH2 and C-SH2) domains in SH-PTP1 have different functions in regulation of the PTPase domain and thereby signal transduction. While the N-terminal SH2 domain is both necessary and sufficient for autoinhibition through an intramolecular association with the PTPase domain, truncation of the C-SH2 domain [SH-PTP1(ΔCSH2) construct] has little effect on SH-PTP1 activity. A synthetic phosphotyrosine residue (pY) peptide derived from the erythropoietin receptor (EpoR pY429) binds to the N-SH2 domain and activates both wild-type SH-PTP1 and SH-PTP1(ΔCSH2) 60- to 80-fold. Another pY peptide corresponding to a phosphorylation site on the IgG Fc receptor (FcγRIIB1 pY309) associates with both the C-SH2 domain (K_d = 2.8 μM) and the N-SH2 domain (K_d = 15.0 μM) and also activates SH-PTP1 12-fold. By analysis of the effect of the FcγRIIB1 pY309 peptide on SH-PTP1(ΔCSH2), SH-PTP1(R30K/R33E), SH-PTP1(R30K/R136K), and SH-PTP1(R136K) mutants in which the function of either the N- or C-SH2 domain has been impaired, we have determined that both synthetic pY peptides stimulate SH-PTP1 by binding to its N-SH2 domain; binding of pY ligand to the C-SH2 domain has no effect on SH-PTP1 activity. We propose that the N-terminal SH2 domain serves both as a regulatory domain and as a recruiting unit, whereas the C-terminal SH2 domain acts merely as a recruiting unit.**

Src homology 2 (SH2) domains are structures of ≈100 amino acids that bind phosphotyrosine residues (pYs) (1). Many SH2-containing protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases) use their SH2 domains to dock on pY loci prior to phosphorylating or dephosphorylating their target proteins. A particularly intriguing class of signaling molecules is the SH2 domain-containing PTKs and PTPases. These molecules, when recruited to new pY loci, could either catalytically amplify or terminate the pY-mediated signaling pathways. The subfamily of SH2-containing cytoplasmic PTPases includes SH-PTP1 (also known as PTP1C, HCP, and SHP) (2–5), SH-PTP2 (also known as PTP2C, PTP1D, and Syp) (6–9), and the *Drosophila* homolog of SH-PTP2, the corkscrew (*csw*) gene product (10). These macromolecules each contain two SH2 domains N-terminal to their PTPase domain. SH-PTP1 is predominantly expressed in cells of hematopoietic origin, whereas SH-PTP2 and corkscrew are more ubiquitously expressed.

SH-PTP2 was shown to associate with autophosphorylated platelet-derived growth factor (PDGF) receptor (7, 8, 11), epidermal growth factor (EGF) receptor (8, 11), and insulin receptor substrate 1 with its SH2 domains (12). SH-PTP1 has been reported to bind c-kit (13), the interleukin (IL)-3 receptor β chain (14), the erythropoietin (Epo) receptor (EpoR) at pY429 (15), and the B-cell IgG Fc receptor II (FcγRIIB1) at pY309 (16). In both enzymes, the N-terminal SH2 domain serves as the autoinhibitory domain for the PTPase domain (17–19). Removal of the N-SH2 domains or occupancy of the SH2 domain(s) by specific pY peptides relieves this autoinhibitory effect of the SH2 domain(s) (17–19). SH-PTP1 has been shown to act as a negative regulator during signaling through the EpoR (15) and the Fc receptor (16), presumably through dephosphorylating pY proteins associated with these receptors.

We have previously shown that resting state autoinhibition of SH-PTP1 by its SH2 domain(s) is mediated by pY-independent intramolecular interaction between the SH2 domain(s) and the PTPase domain (17). We now report that the two SH2 domains in SH-PTP1 have different functions in PTPase regulation and signal transduction. While the N-SH2 domain is both necessary and sufficient for autoinhibition and ligand-induced activation of SH-PTP1, the C-SH2 domain plays little role in this process. Binding of a high-affinity pY ligand derived from the Fc receptor to the C-SH2 domain exerts only a slight effect on SH-PTP1 activity.

MATERIALS AND METHODS

Materials. pY peptides were prepared by solid-phase synthesis using HMP resin (Applied Biosystems) and fluorenylmethoxycarbonyl/2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/1-hydroxybenzotriazole hydrate (FMOC/HBTU/HOBt) chemistry on a 0.15-mmol scale with a Milligen 504 shaker (20). All chemicals were purchased from commercial suppliers and used without further treatment. Crude peptides were purified by reverse-phase HPLC and their identities were confirmed by laser desorption mass spectrometry. Peptide concentrations were determined by digesting to completion with alkaline phosphatase and measuring the amount of inorganic phosphate released by malachite green assay (21).

Bacterial Protein Expression and Purification. Wild-type human SH-PTP1 and its mutants [SH2(N+C), SH2(N+C/R30K/R136K), SH2(N), and R30K/R136K] were expressed in *Escherichia coli* and purified either as a maltose-binding protein (MBP) fusion protein by using the pMAL-C2 expression vector or in their native forms by using pET expression vector systems, as described previously (17, 22). Plasmid pET-SHPTP1(ΔCSH2), which encodes the C-SH2-deleted SH-PTP1 (amino acids 1–106 and 212–595), was generated by sequential treatment of plasmid pET-SHPTP1 (22) with *Spe* I, *Bsp*MI (partial digestion), the Klenow fragment of DNA polymerase I, and T4 DNA ligase. To express SH-PTP1(ΔC60/C453S), the larger *Eag* I–*Eag* I fragment of pET-SHPTP1(ΔC60) (23) was ligated to the smaller *Eag* I–*Eag* I fragment of pET-

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Abbreviations: SH2, Src homology 2; pY, phosphotyrosine residue; PTPase, protein tyrosine phosphatase; Epo, erythropoietin; EpoR, Epo receptor; FcγRIIB1, B-cell IgG Fc receptor II; MBP, maltose-binding protein; *p*NPP, *p*-nitrophenyl phosphate.

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SHPTP1(C453S) (17) to generate plasmid pET-SHPTP1(ΔC60/C453S). To express the C-SH2 as an MBP fusion protein [MBP-SH2(C)], the 1.7-kb *Spe* I–*Hin*dIII fragment of pMAL-SH2(N+C) (17) was inserted into *Xba* I/*Hin*dIII-linearized plasmid pMAL-C2 (New England Biolabs) to give the plasmid pMAL-SH2(C). R30K/R33E and R136K mutants were constructed with oligonucleotide primers by using the method of Kunkel (24). All mutations were confirmed by dideoxynucleotide sequencing. Fig. 1 is a schematic representation of these constructs.

E. coli BL21(DE3) or DH5α cells carrying the appropriate plasmids were grown, induced, and lysed as described (22). MBP fusion proteins were purified on an amylose column (New England Biolabs) as described (25). R30K/R33E, R136K, and ΔCSH2 mutants were purified by following the procedure described for wild-type SH-PTP1 (22), whereas purification of SH-PTP1(ΔC60/C453S) was performed as described for SH-PTP1(ΔC60) (23). Protein concentrations were determined according to Bradford (26), using bovine serum albumin (Bio-Rad) as standard.

PTPase Assay. Phosphatase activity of SH-PTP1 and mutants toward *p*-nitrophenyl phosphate (*p*NPP) was measured at 23°C in a 50-μl reaction mixture containing 10 mM *p*NPP as substrate, 100 mM Hepes (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, 0–600 μM pY peptide as SH2 domain binding ligand, and 0.25 μg of PTPase. After 30 min at 23°C, the reaction was quenched with 950 μl of 1 M NaOH, and the absorbance at 405 nm was measured. In all cases the substrate-to-product conversion was less than 10%.

Binding of pY Peptides to SH2 Domains. All equilibrium binding studies were performed on a Pharmacia BIAcore instrument using CM5 sensor chips provided by Pharmacia, as described (17, 27).

RESULTS

Expression and Purification of SH-PTP1 Mutants. Wild-type SH-PTP1 and mutants were expressed in *E. coli* either in their native forms or as MBP fusion proteins. All proteins were purified to near homogeneity as described in *Materials and Methods* (17, 22). SDS/PAGE analysis showed that all proteins migrate as single bands with the expected molecular weights (data not shown).

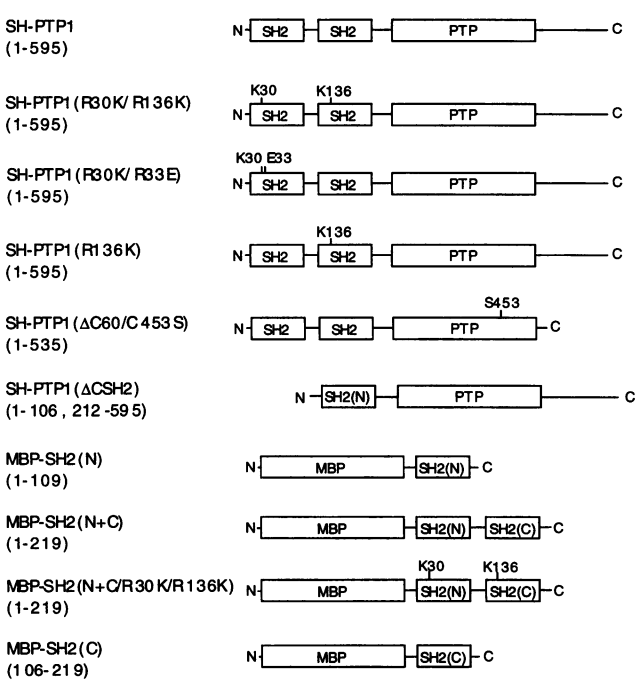

FIG. 1. Structures of constructs used in this work. The numbers in parentheses indicate the starting and ending points within the SH-PTP1 protein used for each construct, according to the numbering scheme of Plutzky *et al.* (3).

N-Terminal SH2 Domain Is Necessary and Sufficient for Autoinhibition/Activation. To gain further insight into the function of the C- and N-terminal SH2 domains, we constructed an internal deletion (residues 107–211) mutant of SH-PTP1 lacking the C-terminal SH2 domain [SH-PTP1(ΔCSH2)] (Fig. 1). This mutant shares many properties of the full-length SH-PTP1. In the absence of stimulatory peptides, its basal activity toward *p*NPP (10 mM) at pH 7.4 is 96.6 nmol/mg per min, slightly lower (83%) than that of full-length SH-PTP1 [95.3 nmol/mg per min, correcting for the lower molecular mass of SH-PTP1(ΔCSH2) (56 kDa vs. 68 kDa for wild type)]. Like full-length SH-PTP1, SH-PTP1(ΔCSH2) can be stimulated by the addition of the EpoR pY429 peptide (DPPHLKpYLYLVVSDSK), which specifically binds to the N-terminal SH2 domain (17). Stimulation of this mutant is concentration dependent; half-maximal activation occurs at ≈40 μM peptide and the maximal activation of 80-fold is reached at 60 to 70 μM peptide (Fig. 2*A*). Under the same conditions, maximal activation of the full-length enzyme is ≈60-fold, 2-fold higher than what we reported previously (17). This small discrepancy in the magnitude of stimulation is likely due to the use of a modified pY peptide (DPPHLKpY-LYLVVSDSK in this work vs. Ac-PHLKpYLYLVVSDK in

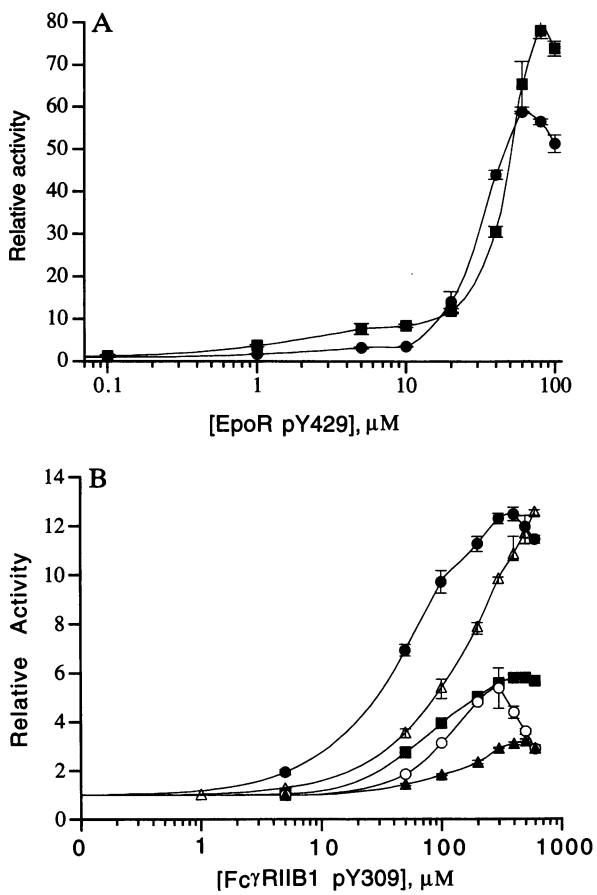

FIG. 2. Concentration dependencies of activation. (*A*) SH-PTP1 (■) or SH-PTP1(ΔCSH2) (●) with EpoR pY429 peptide. (*B*) SH-PTP1 (●), SH-PTP1(R136K) (△), SH-PTP1(ΔCSH2) (■), SH-PTP1(R30K/R136K) (○), or SH-PTP1(R30K/R33E) (▲) with FcγRIIB1 pY309 peptide. Experiments were carried out in HBS buffer (pH 7.4) with *p*NPP (10 mM) as substrate and in the presence of increasing amounts of peptides. The data presented are the mean ± SD values of initial rates from three parallel experiments, relative to the basal activity of SH-PTP1.

the previous one), which has dramatically improved solubility under the assay conditions (pH 7.4). When both enzyme forms are fully activated at 70 μM EpoR pY429 peptide, they show essentially equal catalytic activity. These results suggest that the N-terminal SH2 domain is sufficient both for autoinhibition and for activition by binding to pY peptides.

D'Ambrosio *et al.* (16) have recently reported that SH-PTP1 associates with the cytoplasmic domain of IgG Fc receptor (FcγRIIB1) on B cells, and that a 13-amino acid peptide derived from this receptor (EAENTITpYSLLKH; pY309) specifically associates with the C-SH2 of SH-PTP1 and activates the enzyme by 5-fold *in vitro*. We tested whether this peptide can activate SH-PTP1(ΔCSH2) and full-length SH-PTP1 to the same extent, in an attempt to understand its mechanism of activation. Both enzyme forms are stimulated by the FcγRIIB1 peptide in a concentration-dependent manner (Fig. 2*B*). Full-length enzyme was maximally stimulated 12-fold at ≈400 μM peptide, with half-maximal activation reached at ≈50 μM peptide. SH-PTP1(ΔCSH2) is maximally stimulated 6-fold at 600 μM peptide, with half-maximal activation at ≈50 μM peptide. Note that the concentration of FcγRIIB1 pY309 peptide required to achieve maximal activation of SH-PTP1 is 200-fold higher than the K_d value for the binding of C-SH2 to this peptide (see below), whereas for the EpoR pY429 peptide, half-maximal activation is achieved at 60 μM peptide, 30-fold higher than the K_d value for N-SH2 and the peptide. These results raised the possibility that the FcγRIIB1 pY309 peptide actually activates SH-PTP1 and SH-PTP1(ΔCSH2) by binding to the N-SH2 domain.

To test the above hypothesis, we introduced point mutations into either or both N- and C-SH2 domains, to impair their binding to pY peptides. Structural studies with SH2 domains of Src, Abl, Lck, and SH-PTP2 have established that a universally conserved arginine residue at the βB5 position forms two hydrogen bonds with the negatively charged phosphate group of the bound pY peptide (28–31). Mutation of this arginine to a lysine severely weakened the binding of Abl SH2 domain to pY ligands (32). Similar arginine → lysine mutations in SH-PTP2 also resulted in partial loss of pY peptide-mediated activation of that PTPase (19). In SH-PTP1, this conserved arginine is at position 30 and 136 for the N- and C-terminal SH2 domains, respectively (3). Consistent with the above results, although mutation of R136 to a lysine abolished the binding of C-SH2 to the FcγRIIB1 pY309 peptide (see below), it has minimal effect on the activation of SH-PTP1 by the pY peptide. The activation profile of SH-PTP1(R136K) by the FcγRIIB1 pY309 peptide is very similar to that of wild-type SH-PTP1, although the activation curve for the mutant is slightly shifted toward higher concentrations of peptide, for reasons as yet unclear (Fig. 2*B*). On the other hand, mutation of arginines 30 and 33 in the N-SH2 [SH-PTP1(R30K/R33E)], or the arginine residues in both SH2 domains [SH-PTP1(R30K/R136K)], dramatically decreased the magnitude of activation of these mutant enzymes by the FcγRIIB1 pY309 peptide, with maximal activation of 3- and 6-fold, respectively (Fig. 2*B*). These results therefore establish that activation of SH-PTP1 by FcγRIIB1 pY309 peptide is due to its binding to the N-SH2 domain.

Binding of FcγRIIB1 pY309 Peptide to SH-PTP1. Binding experiments were conducted on a Pharmacia BIAcore biosensor, which utilizes surface plasmon resonance (SPR) to detect binding in real time (33). The FcγRIIB1 pY309 peptide (EAENTITpYSLLKH) was immobilized, through its primary amine group on the lysine residue or the N terminus, onto a carboxymethyldextran polymer attached to a gold-coated surface within a flow chamber. Solutions containing SH-PTP1 or its SH2 domains were passed over the surface, and the amount of protein associated with the immobilized peptide was determined by measuring the SPR signal (in response unit, RU). Under the conditions used, the amount of bound protein is directly proportional to the RU. Mutation of the catalytic cysteine at position 453 was necessary to prevent PTPase-mediated degradation of the immobilized pY peptide, whereas removal of the last 60 amino acids in the mutant greatly reduces nonspecific binding of the protein to the negatively charged carboxymethyldextran surface (17). Addition of free FcγRIIB1 pY309 peptide (50 μM) to the SH-PTP1(ΔC60/C453S) solution prior to injection abolished its binding to the surface (data not shown), indicating that binding of SH-PTP1(ΔC60/C453S) to the surface is due to specific SH-PTP1–pY peptide association. To determine the binding constant of SH-PTP1 for the FcγRIIB1 pY309 peptide, SH-PTP1(ΔC60/C453S) protein at various concentrations was passed over the BIAcore sensor chip, and the steady-state level of bound protein at each concentration was measured by Scatchard analysis (Fig. 3). The dissociation constant (K_d) estimated by curve fitting for these data is 11.7 nM (Table 1).

To obtain direct evidence that the FcγRIIB1 pY309 peptide binds to the SH2 domains of SH-PTP1, we expressed the N-, C-, and N+C-terminal SH2 domains as MBP fusion proteins [MBP-SH2(N), MBP-SH2(C), and MBP-SH2(N+C), respectively (Fig. 1)]. The affinity-purified proteins were then used for binding studies. MBP-SH2(N+C), which contains both SH2 domains, strongly binds to the pY peptide with a K_d of 227 nM (Table 1). However, MBP-SH2(N+C/R30K/R136K), in which the two conserved arginine residues (R30 and R136) expected to bind the phosphate group in an SH2–pY complex are mutated to lysine residues, shows greatly attenuated affinity to the FcγRIIB1 pY309 peptide. Injection of this protein at 24 μM produced no detectable binding, indicating a K_d much greater than 24 μM (data not shown). This is consistent with the above activation results that mutation of these two arginine residues drastically reduces the magnitude of stimulation by the FcγRIIB1 peptide.

In agreement with the findings of D'Ambrosio *et al.* (16), the C-SH2 of SH-PTP1 [MBP-SH2(C)] binds to the FcγRIIB1 peptide, with a K_d of 2.8 μM (Table 1). This binding constant is similar to that of the N-SH2 binding to the EpoR pY429 peptide (1.8 μM) (17) but somewhat higher than the K_d values reported for other SH2-pY complexes (27, 34, 35). We have found that FcγRIIB1 pY peptide also binds to the N-terminal SH2 [MBP-SH2(N)] as well, although the affinity is approximately 1/6 (K_d = 15.0 μM; Table 1). This result shows that FcγRIIB1 pY309 is not a completely specific ligand for the C-terminal SH2 domain of SH-PTP1. It also explains our finding that the FcγRIIB1 pY309 peptide stimulates SH-PTP1 mutants which do not have functional C-SH2 domain

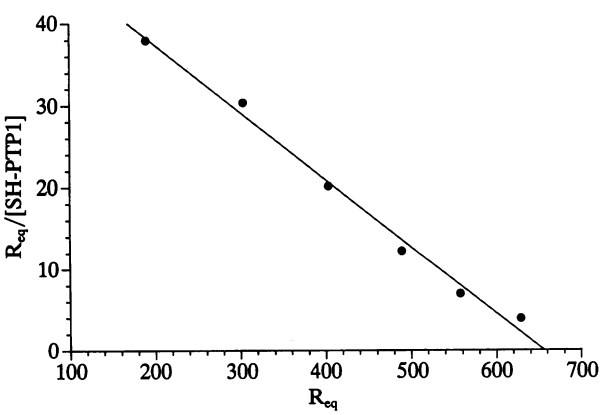

FIG. 3. Scatchard analysis of binding of SH-PTP1(ΔC60/C453S) to FcγRIIB1 pY309 peptide. Various concentrations of SH-PTP1(ΔC60/C453S) (5, 10, 20, 40, 80, and 160 nM) were passed over FcγRIIB1 pY309 peptide surface at a flow rate of 5 ml/min for 4 min. Plotted is R_{eq}/[SH-PTP1(ΔC60/C453S)] vs. R_{eq}, where R_{eq} is the change of resonance signal.

Table 1. Binding constants of pY peptides to SH-PTP1 and its SH2 domains

Protein	K_d, μM	
	FcγRIIB1 pY309	EpoR pY429
MBP-SH2(N)	15.0 ± 3.1	1.8*
MBP-SH2(C)	2.81 ± 0.44	ND
MBP-SH2(N+C)	0.227 ± 0.015	ND
MBP-SH2(N+C/R30K/R136K)	>>24	ND
SH-PTP1(ΔC60/C453S)	0.0117 ± 0.0004	ND

All values reported here (mean ± SD) are from three or more sets of independent measurements. ND, not determined.
*From ref 17.

[(SHPTP1(R136K)] or completely lack the C-SH2 domain [SHPTP1(ΔCSH2)].

DISCUSSION

SH-PTP1 and SH-PTP2 represent an intriguing subclass of PTPases. The presence of SH2 domains in these phosphatases enables their involvement in the signal transduction pathways by means of binding to specific sites of tyrosyl-phosphorylated proteins. SH-PTP1 has been reported to associate with c-kit (13), the interleukin-3 receptor β chain (14), EpoR pY429 (15), and B-cell Fcγ receptor pY309 (16). SH-PTP2 has also been shown to associate with the activated platelet-derived growth factor receptor (7, 8, 11), epidermal growth factor receptor (8, 11), and insulin receptor substrate 1 (12). We (17) and others (18) have previously demonstrated that the N-terminal SH2 domain of SH-PTP1 autoinhibits its phosphatase activity and imposes nonsaturation kinetics on the phosphatase domain. Removal of either the N-SH2 or both N- and C-SH2 domains produces a constitutively activated enzyme which obeys classical saturation kinetic behavior. Occupancy of the N-SH2 domain by a specific pY peptide derived from pY429 of EpoR can also activate SH-PTP1 30- to ≈60-fold and confer saturation kinetic behavior on SH-PTP1 (17). Indeed, recruitment of SH-PTP1 to the EpoR has been shown to cause dephosphorylation and inactivation of JAK2, a protein tyrosine kinase implicated in the EpoR signaling pathway, and myeloid cells expressing mutant EpoR lacking pY429 (these mutants are unable to bind SH-PTP1) are hypersensitive to Epo and display prolonged Epo-induced autophosphorylation of JAK2 (15). In this case the N-SH2 serves both as a regulatory domain and as a recruiting domain, dictating the specificity and activity of SH-PTP1.

Very recently, D'Ambrosio *et al.* (16) have shown that SH-PTP1 associates *in vivo* with the Fc receptor for IgG, an association reportedly mediated by the C-SH2 domain of SH-PTP1 binding to pY309 on the Fc receptor; a 13-amino acid peptide derived from this pY locus (EAENTITpYSLLKH) stimulated the PTPase activity of SH-PTP1 by up to 5-fold. In this work we have provided a quantitative analysis of the association between SH-PTP1 and the FcγRIIB1 pY309 peptide. The C-SH2 domain alone binds to the FcγRIIB1 peptide (K_d = 2.8 μM), in agreement with the qualitative results of D'Ambrosio *et al.* (16). It turns out that the N-SH2 domain alone is also capable of binding to the FcγRIIB1 peptide, although with 1/6 the affinity (K_d = 15.0 μM). The N- and C-SH2 domains together bind the peptide with higher affinity (K_d = 227 nM) than either the N- or C-SH2 domain alone, presumably due to higher effective molarity, which results in increased avidity. SH-PTP1(ΔC60/C453S) binds to the immobilized FcγRIIB1 pY309 peptide with still higher affinity (avidity), with a K_d of 11.7 nM, indicating both N- and C-SH2 domains and the catalytic domain interact with pY peptide molecules which are immobilized on the BIAcore chip surface. It is not yet clear how to relate a BIAcore-derived K_d of 11.7 nM with the 400 μM EC_{50} for SH-PTP1 activity stimulation. For example, the observation that the K_d value for SH-PTP1(ΔC60/C453S) is 1/20 that of MBP-SH2(N+C) could be due to conformational changes in the SH2 domains by addition of a PTPase domain or addition of a third binding site (the PTPase active site). Full-length SH-PTP2 (C459S) also binds to an immobilized platelet-derived growth factor receptor pY1009 peptide with 14-fold higher affinity than the N+C-SH2 domains alone (K_d = 18 nM for SH-PTP2 and 242 nM for N+C-SH2 domain) (36).

Given that FcγRIIB1 pY309 peptide in fact binds to both SH2 domains of SH-PTP1, we turned to deletion and point mutants to assess the role of C-SH2. Deletion of the entire C-SH2 domain, residues 107–211, has almost no effect on the enzyme; both the basal activity and the 80-fold stimulation activity by EpoR pY429 for the ΔCSH2 enzyme are virtually identical to those of wild-type SH-PTP1. Both wild-type SH-PTP1 and SH-PTP1(ΔCSH2) are stimulated by the FcγRIIB1 pY309 peptide, 12-fold and 6-fold for wild-type and ΔCSH2, respectively, which is lower than the 60- to 80-fold activation observed with the N-SH2 binding peptide, EpoR pY429. This is likely due to more effective competitive inhibition at the SH-PTP1 phosphatase active site by the FcγRIIB1 peptide as compared with the EpoR peptide, because much higher concentrations of the FcγRIIB1 peptide are required to achieve maximal stimulation (400–600 μM for FcγRIIB1 pY309 peptide vs. 60–70 μM for EpoR pY429). Both pY peptides can undergo dephosphorylation when treated with SH-PTP1 (ref. 17 and unpublished results). The lower maximal activation of SHPTP1(ΔCSH2) compared with SHPTP1 in the presence of FcγRIIB1 pY309 peptide (12-fold vs. 6-fold) could be due to the possibility that, although the C-SH2 domain plays no role in the enzyme activation, its binding to a pY peptide could induce conformational change that facilitates binding of another pY peptide to the N-SH2 domain to activate the enzyme. In SHPTP1(ΔCSH2), which contains no C-SH2 domain, this fine-tuning mechanism is lifted, and thus the maximal activation would be lower.

These data prove that the FcγRIIB1 pY309 peptide activates SH-PTP1 through interaction with the N-SH2 domain. This view is further supported by the properties of SH-PTP1(R136K), SH-PTP1(R30K/R33E), and SH-PTP1(R30K/R136K). Although the R136K mutation abolishes binding of C-SH2 domain to the FcγRIIB1 pY309 peptide, it has virtually no effect on activation of SH-PTP1 by this peptide. In contrast, the R30K mutation in the N-SH2 domain, while leaving the C-SH2 domain intact, clearly reduces the efficacy of the FcγRIIB1 pY309 peptide in stimulating SH-PTP1. The residual stimulation observed with the R30K mutants can be attributed to the weakened binding of the peptide to the N-SH2 domain. We have previously shown that the R30K mutation does not completely destroy the function of the N-SH2 domain (17), so this residual stimulation is consistent with N-SH2 binding. The binding of FcγRIIB1 pY309 to the C-SH2 domain of SH-PTP1 produces no allosteric relief of phosphatase domain autoinhibition.

Our results suggest two separate mechanisms of action for the two SH2 domains of SH-PTP1 in signal transduction (Fig. 4). In both cases, SH-PTP1 is expected to be a negative regulator and function to terminate the signals. When SH-PTP1 binds its partner protein through its N-terminal SH2 domain, as it does in the case with EpoR signaling (15), SH-PTP1 is both recruited to the partner and activated 30- to 80-fold. The activated enzyme subsequently dephosphorylates the partner protein (e.g., EpoR) or other proteins recruited to the partner protein (e.g., JAK2). Termination of signals in this case would be expected to be rapid and complete, although *in vivo* the rate of termination may well depend on other factors. When SH-PTP1 is recruited to a partner protein through its

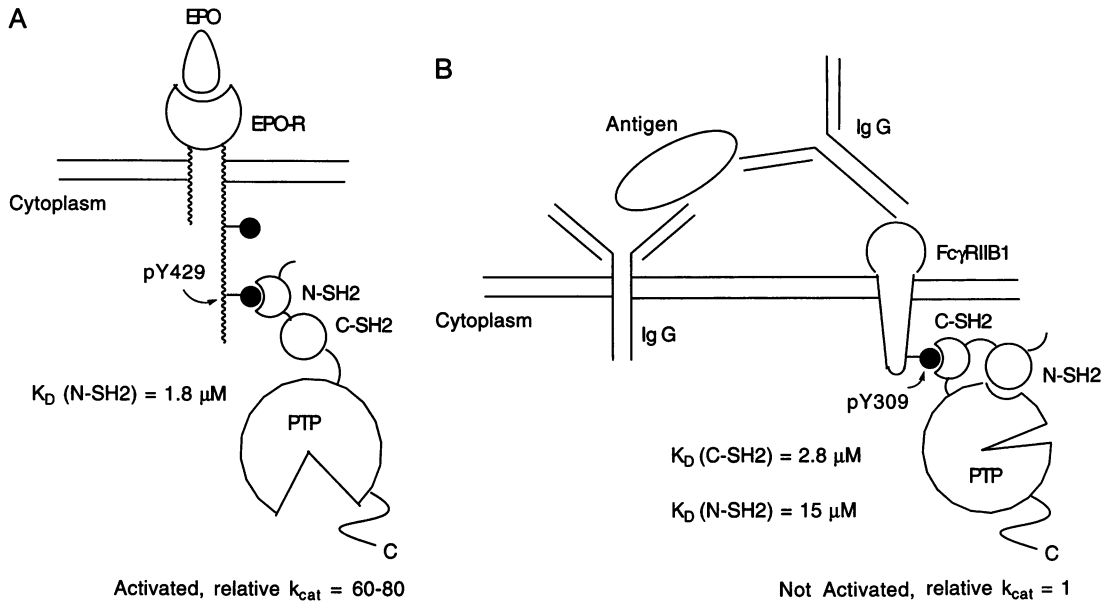

FIG. 4. Mechanism of action of the two SH2 domains. (*A*) SH-PTP1 binds to EpoR at pY429 by means of its N-SH2 domain and thereby relieves autoinhibition of the PTPase domain and shows 60- to 80-fold activation above basal level. (*B*) SH-PTP1 binds to FcγRIIB1 at pY309 by means of its C-SH2 domain. In this case the enzyme is *not* activated.

C-SH2 domain, SH-PTP1 is not activated. Therefore, dephosphorylation of target protein and signal termination might be sluggish and/or incomplete. Indeed, when SH-PTP1 bound to FcγRIIB1 *in vivo*, no change in SH-PTP1 activity was observed (16). An intermediate level of activation could result if pY ligand recruited by C-SH2 domain also binds to some extent to N-SH2 domain [for FcγRIIB1 pY309 peptide, K_d (N-SH2)/K_d (C-SH2) = 6; this ratio would also allow the peptide to equilibrate between the two SH2 domains, should they be close to each other]. In summary, the two SH2 domains of SH-PTP1 appear to have different functions in signaling cascades. While the N-SH2 domain serves both as a regulatory domain and as a recruiting unit, the C-SH2 domain acts chiefly as a recruitment unit.

This work was supported in part by a fund from the Hoffmann–La Roche Institute for Chemistry and Medicine (to C.T.W.). D.P. was a Damon Runyon–Walter Winchell Cancer Research Fund Postdoctoral Fellow (DRG-1172). J.W. is a National Institutes of Health Postdoctoral Fellow.

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