Expression, Purification, and Characterization of SH2-containing Protein Tyrosine Phosphatase, SH-PTP2*

(Received for publication, April 23, 1993, and in revised form, June 16, 1993)

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A human protein tyrosine phosphatase containing two src homology 2 (SH2) domains (SH-PTP2) was expressed in Escherichia coli under T7 promoter control and purified to near homogeneity. The purified protein, with molecular mass of 68 kDa on SDS-polyacrylamide gel electrophoresis, was identified as SH-PTP2 by its protein tyrosine phosphatase activity and N-terminal amino acid sequence analysis. Its protein tyrosine phosphatase activity was sensitive to pH and salt concentration. Whereas its optimum pH for the low molecular weight substrate para-nitrophenyl phosphate is 5.6, the pH optima for peptide substrates were shifted toward neutral. With the artificial protein substrate reduced, carboxyamidomethylated, and maleylated lysozyme, it displays 2000-fold lower $K_m$ (1.7 $\mu$M) and 2.4-fold higher $k_{cat}$ (0.11 s$^{-1}$) than with para-nitrophenyl phosphate. Among the phosphopeptides from autophosphorylation sites of receptors for epidermal growth factor and platelet-derived growth factor, SH-PTP2 displayed high activity toward phosphopeptides corresponding to pY992 of the epidermal growth factor receptor and pY1009 and pY1021 of the platelet-derived growth factor receptor. In further enzymatic studies with phosphopeptides corresponding to pY1009, SH-PTP2 showed nonlinear Lineweaver-Burk double-reciprocal plots, suggesting that the phosphopeptide corresponding to pY1009 may have a substrate and allosteric effect.

Protein tyrosine phosphorylation, regulated by the interplay between protein tyrosine kinases and protein tyrosine phosphatases, is an important mechanism for the control of cell proliferation and differentiation (1–5). Although the structure, function, and regulation of protein tyrosine kinases have been rather well established (6, 7), those of the protein tyrosine phosphatases remain obscure (8–10). So far, more than 30 transmembrane and nontransmembrane protein tyrosine phosphatases have been identified (11–13). Among the nontransmembrane protein tyrosine phosphatases, we have cloned SH-PTP1 (14), also known as PTPL1C (15), HCP1 (16), SHP (17), and PTPLN6 (18) and SH-PTP2 (19), also known as SH-PTP3 (20), PTPL1D (21), and PTPL2 (22), both of which contain two SH2 domains upstream from the conserved catalytic domain. Mouse Syp is reported as a homologue of SH-PTP2 based on the high similarity of the cDNA sequence (23). Rat PTPL1 (24) may be also a homologue of SH-PTP2, based on the high similarity in reported partial amino acid sequences of PTPL1 with SH-PTP2.

SH2 domains are also found in several other types of signaling proteins, such as src family protein tyrosine kinases, GTPase-activating protein, phospholipase C-γ, and p85, the regulatory subunit of phosphatidylinositol 3-kinase (25). SH2 domains bind to tyrosine-phosphorylated sequences in proteins and peptides, thereby facilitating inter- and intramolecular protein-protein interactions, including enzyme-substrate interactions (26). Recently, phosphotyrosine-independent binding to SH2 domains has also been reported (27). The finding of protein tyrosine phosphatases with SH2 domains that would, presumably, target these enzymes to specific phosphotyrosine-containing protein substrates is of distinct physiological interest.

Although SH-PTP1 is predominantly expressed in hematopoietic cells (14, 16, 17), SH-PTP1 is ubiquitously expressed (19, 20, 22). Based on its sequence similarity and comparable expression pattern, SH-PTP2 may be the homologue of the Drosophila corkscREW gene product (Csw) (19, 28). Genetic epistasis experiments indicate that Csw functions in the terminal class signal transduction pathway in concert with the Drosophila c-Raf homologue (illos polehill gene product (29) or D-Raf), to positively transduce signals generated by the torso receptor protein tyrosine kinase (30, 31), a PDGF receptor (PDGFR) homologue.

We and others (21, 23, 32) have shown that SH-PTP2 is tyrosyl-phosphorylated in vivo upon activation of the EGFr receptor (EGFR) or the PDGFR, although this has not been shown to be a direct effect of the receptor kinase. Moreover, we have found that SH-PTP2 is directly bound to EGFR and PDGFR via its N-terminal SH2 domain following ligand activation (32). Since the EGFR (33–35) and the PDGFR (36–40) are protein tyrosine kinases and are autophosphorylated within the cytoplasmic domain, it seems likely that SH2 domains in SH-PTP2 will play a crucial role in binding to the EGFR and PDGFR via their autophosphorylation sites and for further signal transduction. SH-PTP2 binds to phosphorylated

‡ Supported by Damon Runyon-Walter Winchell Cancer Research Fund Postdoctoral Fellowship DRG-062.

** Supported in part by a Junior Faculty Research Award from the American Cancer Society.

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* This work was supported by National Institutes of Health Grants 49152 (to B. G. N.) and GM20011 (to C. T. W.), funds from F. Hoffmann-La Roche Ltd. (to B. G. N. and C. T. W.) and the National Science Foundation (to S. E. S.), and by Joslin Diabetes Center National Institutes of Health DERC Grant 36836. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked § to indicate this fact.

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The abbreviations used are: SH2, src homology 2; EGF, epidermal growth factor; EGFR, EGF receptor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; pNPP, para-nitrophenyl phosphate; RCM-lysozyme, reduced, carboxamidomethylated, and maleylated lysozyme; pY, phosphotyrosyl residue; MES, 2-(N-morpholino)ethanesulfonate; DTI, dithiothreitol; BSA, bovine serum albumin.

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PDGF via C-terminal tyrosine 1009. The effects of phosphor-ylation of SH-PTP2 and binding of SH-PTP2 SH2 domain to receptor autophosphorylation sites(s) on SH-PTP2 enzymatic activity have not been determined.

In this paper, we describe the expression, purification, and enzymatic properties of recombinant SH-PTP2 derived from Escherichia coli, including its interactions with phosphoprotein-273-according to autophosphorylation sites of EGFR and PDGF as protein tyrosine phosphatase substrates and effectors.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases NdeI and SalI were purchased from New England Biolabs and Thal from Life Technologies, Inc. p-NPP was from Sigma. Reduced, carboxymethylated, and maleylated (RCM) lysozyme was from Life Technologies, Inc. v-Ab was from Oncogene Science. The expression vector pET-SHPTP1 (41) was kindly donated by D. Pei (Harvard Medical School). Oligonucleotide adapters and sequencing primers were synthesized by A. Nussbaum (Harvard Medical School). Phosphoprotein-273-EGF-PRF-4 was kindly donated by H. Cho (Harvard Medical School).

Plasmid Construction—A 1.95-kilobase pair Thal-Sal1 fragment encoding amino acid residues 4-503 of SH-PTP2 was isolated from plasmid pBS FB 21-7, which is a cloning vector pBluescript KS (49) containing the entire cDNA of SH-PTP2 (19). The N-terminal 3 amino acids were re-introduced using two oligonucleotide adapters, 5'-GATCATCGC-3' (10-mer) and 5'-ACTGATGACG-3' (8-mer), to facilitate subcloning. The 10- and 8-mer adapters and the 1.95-kilobase pair Thal-Sal1 fragment were ligated into NdeI-SalI-linearized PET-SH-PTP1 to generate plasmid pET-SHPTP2. The sequence from the Stu1-Dalgalano sequence to 15th amino acid from the N terminus was confirmed by dideoxy sequencing using the T7 promoter primer, 5'-TATACGACTCACATTAGGG-3' (20-mer).

Expression and Purification of SH-PTP2—E. coli strain BL21 (DE3) transformed with plasmid pET-SHPTP2 was grown in 4 liters of LB medium containing 50 μg/ml ampicillin at 37°C to an absorbance at 595 nm of 0.8 and induced for 3 h at 30°C with 0.4 mM isopropyl-D-thio-β-D-galactopyranoside. Cells were harvested by centrifugation and resuspended in 150 ml of buffer A containing 50 μg/ml aprotinin, 20 μg/ml leupeptin, and 20 μg/ml pepstatin. The cells were disrupted by French Press, and the crude lysate was centrifuged at 15,000 rpm for 15 min in a Sorvall SS-34 rotor. The supernatant was equilibrated with buffer A containing 20% saturated ammonium sulfate, and loaded onto a phenyl-Sepharose (Sigma) column (13.2 × 2.5 cm) equilibrated with buffer A containing 20% saturated ammonium sulfate. The column was washed with the same buffer, and activity was eluted using an ammonium sulfate concentration gradient, 20 to 0%, in 400 ml of buffer A at 0.5 mM/min. Activity, recovered in the flow-through and very early part of gradient-elution, was precipitated by 60% saturated ammonium sulfate, dissolved in buffer A containing 20% saturated ammonium sulfate, and loaded onto a phenyl-Sepharose (Sigma) column (13.2 × 2.5 cm) equilibrated with buffer A containing 50% saturated ammonium sulfate. The column was washed with the same buffer, and activity was eluted using an ammonium sulfate concentration gradient, 20 to 0%, in 400 ml of buffer A at 0.5 mM/min. The active fractions, eluting from 125 to 150 mM NaCl, were concentrated using a 30,000 MWCO membrane and the concentration of the active fractions, eluting at approximately 8% saturated ammonium sulfate, were dialyzed against 10 mM MES, pH 5.7, containing 10 mM 2-mercaptoethanol (buffer B), and divided into four aliquots of equal volume. Each aliquot was loaded onto Mono S HR 10/10 (Pharmacia LKB Biotechnology Inc.) column equilibrated with buffer B. The column was developed with a gradient of 0-250 mM NaCl in 500 ml of buffer B at 2.5 mM/min. Pooled fractions, eluting from 125 to 150 mM NaCl, were concentrated using a Centricon-10 (Amicon) and stored at −80°C in the presence of 33% (v/v) glycerol.

Assay for Protein Tyrosine Phosphatase Activity—With p-NPP as substrate, typically 10 μM p-NPP was incubated with 87 μg/ml SH-PTP2 at 24°C for 140 min in 50 μl of 50 μM 3,3-dimethylglutarate, pH 5.6, containing 10 mM 1,4-NaCl, 10 mM EDTA, and 2 mM EDTA. The reaction was quenched with 950 μl of 1 M NaOH, and the absorbance of p-nitrophenolate at 405 nm was measured. The amount of p-nitrophenol released was calculated by comparison with a standard curve obtained with p-nitrophenol (Sigma).

To assay the dephosphorylation of phosphopeptides, the release of Pn was measured by malachite green assay (42-44). Typically, 500 μM phosphopeptide was incubated with 87 μg/ml SH-PTP2 at 20°C for 12 min in 50 μl of 50 mM HEPES, pH 7.1, containing 150 mM NaCl, 10 mM DTT, and 2 mM EDTA. The reaction was quenched with 950 μl of malachite green reagent, and the absorbance at 650 nm was measured. The amount of released Pn was calculated with a standard curve. Phosphopeptides were synthesized as described in Piccione et al. (45) using the methodology of Kias et al. (46). Sequence of phosphopeptides used here were as follows: EGFR pY992, DADEYLYIPQQQFF; EGFR pY1068, VPEYIQINSQVPK; EGFR pY1086, NVPYHNPGLNLP; EGFR pY1148, NPDpYPYIPPEFPK; EGFR pY1173, TAEANAYLKVDA; PDGFR pY740, DGGpYMDMSKDE; PDGFR pY751, SVdpYVPMCDMK; PDGFR pY771, SSMPYMAPYDNY; PDGFR pY1009, SVLPYATVPQNE; PDGFR pY1021, DNDYpY1F1, PDPKK, with pY indicating the phosphor-ylated tyrosine.

To assay the dephosphorylation of a protein substrate, phosphor-ylated RCM-lysozyme was prepared essentially by the method of Tonks et al. (47), but using v-Ab as the kinase. Typical specific activity obtained was 13 μCl/mmol. Assay conditions were essentially the same as those of Tonks (47). The indicated concentration of phosphor-ylated RCM-lysozyme was incubated with 330 ng/ml SH-PTP2 at 30°C for 5 min in 60 μl of buffer reaction consisting of 25 mM HEPES, pH 7.2, containing 3 mg/ml bovine serum albumin (BSA), 5 mM EDTA, and 10 mM DTT. At 1 and 5 min, 25 μl of reaction mixture was transferred to a suspension of activated charcoal. From the counts in the supernatant, phosphor-ylation velocities were calculated.

Other Methods—The concentration of protein was determined by Bradford assay (Bio-Rad) using BSA as standard (48). SDS-PAGE was carried out as described by Laemmli (49). Gel filtration was carried out with Superose 12 (10/30, Pharmacia) equilibrated with 25 mM HEPES, pH 7.2, containing 200 nM NaCl, at a flow rate of 0.5 ml/min using aldolase (Mr, 158,000), BSA (Mr, 67,000), and ovalbumin (Mr, 43,000) as standards. N-terminal sequencing was performed by the Edman method (50) using an automated gas-phase sequencer (Applied Biosystems).

RESULTS

Expression and Purification of SH-PTP2—Previously we observed that SH-PTP1, a protein highly similar to SH-PTP2, was highly expressed in E. coli and that when expressed in bacterial cells, SH-PTP1 accumulated partially as a soluble protein (41). Therefore, we used the same expression vector with the coding region of SH-PTP2. The expression level of SH-PTP2 was low, approximately 1% of total E. coli cell protein content, with about 10% for SH-PTP1. However, most of the activity was recovered in the soluble fraction (data not shown).

Soluble bacterially expressed SH-PTP2 was purified according to the scheme summarized in Table I. During the purification, p-NPP was used as substrate. E. coli alkaline phosphatase also reacts with p-NPP. During the first three steps, SH-PTP2 is likely contaminated with alkaline phosphatase, suggesting that the yield for the last three steps is higher than the values in Table I. Using this scheme, SH-PTP2 was purified to greater than 90% purity based on SDS-PAGE analysis (Fig. 1). The molecular mass of the purified protein was 68 kDa, consistent with the 593-amino acid sequence and similar to the in vivo molecular mass of 68-70 kDa (21, 23, 32). The purified enzyme behaved as a monomer on gel filtration (data not shown). The N-terminal sequence of the purified protein was analyzed up to the 10th residue and matched the predicted sequence from residue 2 on, indicating processing of the initiating N-terminal methionine.

Biochemical Characterization of SH-PTP2 Phosphatase Activity toward p-NPP—We elucidated the optimum pH, salt concentration, and temperature for SH-PTP2 activity initially using the low molecular weight substrate p-NPP. As shown in Fig. 2, like SH-PTP1 (41) and other protein tyrosine phosphatases (51), SH-PTP2 showed an acidic pH optimum with this substrate, pH 5.6. As determined by the NaCl concentration profile for SH-PTP2, the optimal salt concentration for SH-PTP2 activity toward p-NPP was 50 mM at pH 5.6 with only 9% activity
TABLE I

Summary of SH-PTP2 purification

| Steps                      | Volume | Protein | Activity | Specific activity | -Fold | Yield |
|----------------------------|--------|---------|----------|------------------|-------|-------|
|                            | ml     | mg/ml   | units/ml | units/mg         |       | %     |
| Crude lysate               | 125    | 31.0    | 24       | 0.77             | 1     | 100   |
| Q-Sepharose                | 9.4    | 12      | 1.28     | 1.7              | 80    |
| Ammonium sulfate precipitation | 50   | 37      | 1.95     | 2.5              | 62    |
| Phenyl-Sepharose           | 112    | 0.36    | 2.7      | 7.5              | 9.7   |
| Mono S                     | 60     | 30      | 30       | 39               | 3     |
| Centriprep 10              | 2.25   | 1.33    | 40       | 39               | 3     |

high salt (300 mM) (data not shown).

SH-PTP2 required long reaction times because of its low activity toward p-NPP. To assess the stability of SH-PTP2 under such reaction conditions, we analyzed temperature dependence by Arrhenius analysis. Fig. 3 shows that SH-PTP2 was stable below 24 °C at pH 5.6, even at long reaction times (130 min).

Kinetics of SH-PTP2 toward p-NPP and Phosphotyrosyl-RCM-lysozyme—As a p-nitrophenylphosphatase, SH-PTP2 is a slow enzyme ($k_{cat} = 0.046$ s$^{-1}$), even under optimum pH and ionic-strength conditions (Table II). However, assay with phosphotyrosyl-RCM-lysozyme (Table III) revealed a 2.4-fold higher catalytic activity ($k_{cat} = 0.11$ s$^{-1}$). Concomitantly, $K_m$ drops 2000-fold from p-NPP (3.6 mM) to RCM-lysozyme (1.7 μM). Thus, the use of p-NPP severely underestimates (by some 5000-fold) the catalytic efficiency ($k_{cat}/K_m = 13$ M$^{-1}$ s$^{-1}$ versus $6.5 \times 10^4$ M$^{-1}$ s$^{-1}$) compared with a protein substrate.

Properties of SH-PTP2 toward Phosphopeptide from the EGFR and the PDGFR Autophosphorylation Sites—Given the in vivo evidence for specific binding of SH-PTP2 to EGFR and PDGFR (32), we have evaluated the properties of autophosphorylation site peptides from the cytoplasmic domains of each of these transmembrane growth factor receptors as substrates of and/or effectors for SH-PTP2. An initial screen utilized the synthetic 11–13-amino acid phosphopeptides corresponding to each known autophosphorylation site (33-40). As indicated in Fig. 4A, on incubation at pH 5.6 (the pH optimum determined for p-NPP (see Fig. 2)), the EGFR pY992 was dramatically better as a substrate than the other EGFR autophosphorylation site peptides. When the EGFR pY992 and EGFR pY1173 phosphopeptides were compared (“good” and “bad” substrates, respectively), EGFR pY1173 showed a pH optimum at pH 6.1 (Fig. 2), but the EGFR pY992 substrate showed a pH optimum in the physiological pH range (Fig. 2). Thus, subsequent characterization was done under physiological conditions. At neutral pH, the EGFR phosphopeptides show a similar profile to that at acidic pH (Fig. 4, B versus A) with EGFR pY992 showing >10-fold preferential substrate activity for SH-PTP2.

In comparison with EGFR peptides, the distinction between the five phosphopeptides from the PDGFR at pH 5.6 was less (Fig. 4A); PDGFR pY1021 was chosen as was PDGFR pY1109 for subsequent kinetic evaluation. At physiological pH, PDGFR pY1021 is now preferred about 2-fold over PDGFR pY1021 and 4–10-fold over the other sites (Fig. 4B).
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Table II: Kinetic constants for PTPases for p-NPP

| Enzymes        | \( k_{\text{cat}} \) | \( K_m \) | \( k_{\text{cat}}/K_m \) | Conditions            | Ref. |
|----------------|---------------------|----------|--------------------------|-----------------------|-----|
| YOP51          | 1234                | 2.40     | 514                      | pH 5.0, 100 mM acetate, \( I = 0.15 \) m, 30 °C | 51  |
| PTPu323        | 48                  | 0.23     | 209                      | Same as above         | 51  |
| YPTP1          | 1.6                 | 1.18     | 1.36                     | pH 5.5, 100 mM acetate, \( I = 0.15 \) m, 30 °C | 51  |
| rLAR           | 6.1                 | 0.42     | 14.5                     | pH 5.0, 40 mM MES, 30 °C | 53  |
| LARD1          | 4.1                 | 1.73     | 2.37                     | pH 5.0, 100 mM MES, 25 °C | 54  |
| VH1            | 0.3                 | 7.7      | 0.399                    | pH 5.5, 100 mM acetate, \( I = 0.15 \) m, 25 °C | 51  |
| cdc25          | 0.033               | 50       | 0.00066                  | pH 8.2, 100 mM Tris, 250 mM NaCl, 37 °C | 55  |
| SH-PTP1        | Full length         | 155      | 148                      | pH 5.5, 100 mM MES, 150 mM NaCl, 10 mM DTT, 1 mM EDTA, 23 °C | 41  |
| SH-PTP1        | SH2 domains deleted | 110      | 24-36                    | pH 6.3, 100 mM MES, 150 mM NaCl, 10 mM DTT, 1 mM EDTA, 23 °C | 41  |
| SH-PTP1 (PTP1C)| Full length         | 37.4     | 1.5                      | pH 5.0, 25 mM acetate, 20% glycerol, 1 mM DTT, 1 mM EDTA, 23 °C | 56  |
| SH-PTP2        | Full length         | 0.046    | 3.6                      | pH 5.6, 50 mM 3,3-dimethylglutamate, 50 mM NaCl, 10 mM EDTA | 22  |
| SH-PTP2 (PTP2C)| SH2 domains deleted | 49*      | ND*                      | pH 5.0, 25 mM acetate, 20% glycerol, 1 mM DTT, 1 mM EDTA, 23 °C | 22  |

\( *K_m \) not determined. 49 s^{-1} estimated from reported velocity at a fixed concentration of 10 mM p-NPP (22).

Table III: Kinetic constants for SH-PTPs toward RCM-lysozyme

| Enzymes        | \( k_{\text{cat}} \) | \( K_m \) | \( k_{\text{cat}}/K_m \) | Conditions            | Ref. |
|----------------|---------------------|----------|--------------------------|-----------------------|-----|
| PTP1C          | Full length         | 0.14     | 1.9                      | pH 7.0, 25 mM imidazole HCl, 1 mM DTT, 1 mM EDTA, 37 °C | 51  |
| SH-PTP2        | Full length         | 0.11     | 1.7                      | pH 7.2, 25 mM HEPES, 10 mM DTT, 5 mM EDTA, 1 mg/ml BSA, 30 °C | 56  |
| SH-PTP2 (PTP2C)| SH2 domains deleted | 0.35*    | ND*                      | pH 7.0, 25 mM imidazole HCl, 1 mM DTT, 1 mM EDTA, 1 mg/ml BSA, 30 °C | 22  |

\( *K_m \) not determined. 0.35 s^{-1} estimated from reported velocity at a fixed concentration of 1 μM RCM-lysozyme (22).

As mentioned above, our unpublished data indicate that SH-PTP2 binds to phosphorylated PDGF-R via C-terminal tyrosine 1009, suggesting that PDGFR pY1009 may both regulate and act as substrate for the protein tyrosine phosphatase activity of SH-PTP2. Therefore, we chose PDGFR pY1009 for further analysis with pure SH-PTP2. Deviations from simple Michaelis-Menten hyperbolic saturation behavior were detected. Fig. 5 shows a Lineweaver-Burk plot of PDGF-R pY1009 which reproducibly shows curvature. A simple linear analysis from data at low substrate concentration would yield a y axis crossing in the negative region, which precludes calculation of the \( K_m \) and \( k_{\text{cat}} \). However, for data at higher substrate concentration, the slope approaches linear behavior, and an extrapolated line gave a \( V_{\text{max}} \) estimate of 20 μmol/min (inset of Fig. 5). It may be that this peptide shows both allosteric and substrate effects.

**DISCUSSION**

In this work, the full-length human SH2 domain-containing protein tyrosine phosphatase, SH-PTP2, was expressed in E. coli and highly purified. Basic enzymatic properties have been assessed with a low molecular weight substrate p-NPP and subsequently with 11-13 residue phosphopeptides and tyrosine-phosphorylated RCM-lysozyme.

Protein tyrosine phosphatase activity of SH-PTP2 toward p-NPP shows an acidic pH optimum and sensitivity to ionic strength, similar to SH-PTP1 (41). From the Arrhenius plot (Fig. 3), SH-PTP2 was stable for 130 min at pH 5.6 below 24 °C, but was labile above 24 °C. Although assays with RCM-lysozyme were done at 30 °C for 5 min, our data were within linear regions of Pi release versus time, indicating no inactivation during the reaction.

In Table II, \( K_m \) and \( k_{\text{cat}} \) values for p-NPP are compared with those reported for other protein tyrosine phosphatases (22, 41, 51, 53-56). As a p-nitrophenolphosphatase, SH-PTP2 is dramatically slow, even under optimum pH and ionic strength conditions. It has 30,000-fold lower \( k_{\text{cat}} \) than the Yersinia protein tyrosine phosphatase Yop (51). The \( k_{\text{cat}} \) of SH-PTP2 as a p-nitrophenolphosphatase is lower than that of VH1 (51) and slightly higher than that of cdc25 (55). At 10 mM p-NPP, SH-PTP2 has about 2% the catalytic activity of the highly similar, E. coli-expressed, SH-PTP1 (41 and data not shown). To determine that SH-PTP2 expressed was not largely inactive, we compared the activity of SH-PTP2 toward phosphotyrosyl-RCM-lysozyme with that of SH-PTP1 (PTP1C) (56) and with the value just reported for SH2 domain-deleted SH-PTP2 mutant (ΔSH2-PTP2C) (22) (Table III). Kinetic data for SH-PTP2 are almost the same as those of SH-PTP1 (PTP1C) (56), and the \( k_{\text{cat}} \) of SH-PTP2 is about one-third the value of the SH2 domain-deleted SH-PTP2 mutant (22). We also compared the activity of SH-PTP2 toward the phosphotyrosyl peptides EGFR pY992 and EGFR pY1173 with SH-PTP1 expressed in E. coli (41). SH-PTP2 is only 5-8-fold slower than SH-PTP1 under the same assay conditions (data not shown). These comparisons are consistent with the view that the purified SH-PTP2 is correctly folded full-length enzyme. It may be worthwhile to express full-length SH-PTP2 in a eukaryotic overproduction system (e.g., baculovirus) to compare catalytic efficiency.

Since SH-PTP2 has SH2 domains and protein tyrosine phosphatase domain, it is possible that phosphotyrosyl proteins serve as both substrates and SH2 domain targets. Using phosphotyrosyl peptide substrates from the EGFR (33-35) and PDGFR (36-40) cytoplasmic domains, SH-PTP2 shows high activity for three of the 10 autophosphorylation site peptides (Fig. 4). For PDGFR pY1009 which has been examined carefully, the anomalous dependence of velocity on pY peptide concentration may reflect both allosteric and active site binding. Songyang et al. (57) selected the sequence motif of pY-VI-X-V.
over, we recently found that pY1009 is the binding site in domains derived from SH-PTP2. Initial binding studies of PDGFR for glutathione S-transferase fusion proteins with SH2 N-terminal SH2 domain of SH-PTP2. PDGFR pY1009 has the sequence pYTAV, approximating the enriched sequence. More-

from a synthetic peptide library as an enriched ligand for the receptor phosphotyrosine. Assays were done under the same conditions described under "Experimental Procedures," at pH 5.6, was used instead of 50 mM HEPES buffer (A).

Limited tryptic cleavage of the 68-kDa intact SH-PTP2 to yield a 65-kDa fragment also increases protein tyrosine phosphatase activity by four-fold. Similarly, after partial tryptic digestion resulting in cleavage of the C-terminal 5-kDa fragment, SH-PTP1 (PTP1C) enzymatic activity increased about 103-fold higher than full-length SH-PTP2 (0.046 s⁻¹) (Table II). Toward RCM-lysozyme, the velocity of ASH2-PTP2C (0.35 s⁻¹) (22) is only 3-fold higher than full-length SH-PTP2 (0.11 s⁻¹) (Table III). We recently (41) reported that deletion of SH2 domains from SH-PTP1 increases the catalytic efficiency toward p-NPP, but only 3-5-fold, from 1.0 x 10³ m⁻¹ s⁻¹ to 2.9-4.6 x 10³ m⁻¹ s⁻¹ (Table II). These comparisons of kinetic data between full-length and SH2 domain-deleted SH-PTP1 and SH-PTP2 imply negative regulation of 5-1000-fold of protein tyrosine phosphatase activity by unoccupied SH2 domains.}

Limited tryptic cleavage of the 68-kDa intact SH-PTP2 to yield a 65-kDa fragment also increases protein tyrosine phosphatase activity by four-fold. Similarly, after partial tryptic digestion resulting in cleavage of the C-terminal 5-kDa fragment, SH-PTP1 (PTP1C) enzymatic activity increased about 103-fold (56). We have not confirmed the cleavage site yet for SH-PT2, but if tryptic digestion of SH-PT2 also removes the C-terminal region, then this would be an indication that the C-terminal tail of SH-PT2, as in SH-PTP1, also has negative autoregulatory effect.

So far we have noted the stimulatory or inhibitory effects of phosphopeptides from receptor kinases and SH2 domains and the C-terminal regions of SH-PTPs. It is important, we believe,
to clarify the relationship of these effects. To resolve these issues, we plan to compare the properties of SH2 and C-terminal domain-deleted or -substituted mutants and examine the contribution of each domain to both binding and catalytic activities using phosphorylated and nonphosphorylated peptides or proteins.

During the preparation of this manuscript, two papers have appeared, one describing the in vivo characterization of SH-PTP2 in a transient expression system (21) and another characterizing the murine homologue of SH-PTP2 (22). In the former paper, SH-PTP2 (named PTPID by these authors) was co-transfected into 293 cells with receptor chimeras composed of the extracellular domain of the EGFR and cytoplasmic domain of the PDGFR. Immunoprecipitated SH-PTP2 (PTPlD) from ~-[~S]Met-labeled 293 cell transfectants was analyzed with or without stimulation by EGF. On EGF stimulation, the phosphorylation level of SH-PTP2 was elevated and protein tyrosine phosphatase activity was increased slightly (1.2-fold). The contribution of phosphorylation and SH2 domain binding to this increase in phosphatase activity is unclear. Experiments are currently under way in our labs to clarify these issues.

Acknowledgments—We thank H. Cho for phosphopeptide preparation, D. Pei for expression plasmid, and the Dana Farber Cancer Institute and Harvard Microchemistry Facility for N-terminal amino acid sequencing analysis.

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