Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1092–1096, February 1993
Biochemistry

Overexpression, purification, and characterization of SHPTP1, a Src homology 2-containing protein-tyrosine-phosphatase

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ABSTRACT **A protein-tyrosine-phosphatase (PTPase; EC 3.1.3.48) containing two Src homology 2 (SH2) domains, SHPTP1, was previously identified in hematopoietic and epithelial cells. By placing the coding sequence of the PTPase behind a bacteriophage T7 promoter, we have overexpressed both the full-length enzyme and a truncated PTPase domain in *Escherichia coli*. In each case, the soluble enzyme was expressed at levels of 3–4% of total soluble *E. coli* protein. The recombinant proteins had molecular weights of 63,000 and 45,000 for the full-length protein and the truncated PTPase domain, respectively, as determined by SDS/PAGE. The recombinant enzymes dephosphorylated *p*-nitrophenyl phosphate, phosphotyrosine, and phosphotyrosyl peptides but not phosphoserine, phosphothreonine, or phosphoseryl peptides. The enzymes showed a strong dependence on pH and ionic strength for their activity, with pH optima of 5.5 and 6.3 for the full-length enzyme and the catalytic domain, respectively, and an optimal NaCl concentration of 250–300 mM. The recombinant PTPases had high K_m values for *p*-nitrophenyl phosphate and exhibited non-Michaelis–Menten kinetics for phosphotyrosyl peptides.**

Protein-tyrosine phosphorylation is a common mechanism by which growth factors (GFs) regulate cellular proliferation and differentiation (1). Many GF receptors are transmembrane protein-tyrosine kinases (PTKs), which cluster and transphosphorylate themselves upon ligand binding. Activated GF receptors subsequently transmit signals by physically associating with and/or phosphorylating downstream signaling molecules. In normal cells, the levels of tyrosine phosphorylation and thus the extent and duration of the signals transmitted are regulated by the presence of another family of enzymes, protein-tyrosine-phosphatases (PTPases; EC 3.1.3.48), which remove phosphates from phosphotyrosine residues. Indeed, oncogenic transformation is often due to increased levels of PTK activity (1, 2).

A large number of PTPases have been identified through molecular cloning, and they can be grouped into transmembrane and intracellular families (3). Transmembrane PTPases, such as CD45 (4) and the leukocyte antigen-related (LAR) PTPase (5), with intracellular catalytic domains and unrelated extracellular domains resemble in structural organization several transmembrane tyrosine kinases such as the epidermal growth factor receptor (EGFR); these receptor-like PTPases may be capable of initiating transmembrane signaling in response to as-yet-unidentified ligands, presumably by modulating their PTPase activity. The regulation and function of intracellular PTPases are largely unknown. Recently, several laboratories have identified a subfamily of intracellular PTPases, including SHPTP1 (6–9), *Drosophila* corkscrew gene product (10), and SHPTP2 (11). These three PTPases show extensive sequence similarity (52–76% identity; ref. 11) and they each contain two Src homology 2 (SH2) domains N-terminal to their PTPase domain. SH2 domains are conserved stretches of approximately 100 amino acids, originally identified in the Src-like intracellular PTKs and subsequently found in a variety of other signaling molecules (12). SH2 domains specifically bind phosphotyrosyl proteins and promote intra- and intermolecular protein–protein interactions. Different subsets of phosphotyrosyl proteins bind to different SH2 domains with various affinities. The specificity of the cellular response to GFs may be largely determined by the strength and spectrum of the intermolecular interactions between the SH2 domains of secondary signaling molecules and tyrosyl autophosphorylated GF receptors (12). SH2 domains of Src-like kinases are also known to modulate their own kinase activity (1, 12). The presence of SH2 domains in PTPases raises some interesting possibilities about the structure and function of the SHPTPs (6). First, these SHPTPs, like SH2-containing secondary signaling molecules, may bind autophosphorylated GF receptors, thereby being actively involved in the signal transduction pathway. Second, the SH2 domains conceivably could bind their own C-terminal tails (the C termini of all three SHPTPs each contains three conserved tyrosine residues) and negatively regulate their phosphatase activity. However, the detailed characterization of these SHPTPs has been hampered by the lack of purified enzymes. To begin to evaluate the possible roles of the SH2 domains on specificity and catalytic activity of the PTPases, it is desirable to overexpress these proteins and isolate them in sufficient quantities and pure form.

We report here the expression in *Escherichia coli* of both the full-length human SHPTP1 and its catalytic domain lacking the N-terminal SH2 domains, their purification, and their initial characterization. We show that both enzymes have an absolute specificity toward phosphotyrosine residues, and their specific activities are extremely sensitive to ionic strength and pH conditions. The enzymes bind small substrates with very low affinities and show anomalous kinetics with synthetic phosphotyrosyl peptides.

MATERIALS AND METHODS

Materials. Restriction endonucleases and DNA-modifying enzymes were purchased from New England Biolabs. Expression vectors pGEX-3Xb and pT7-7 were kindly provided by G. Payne and S. Tabor (Harvard Medical School), respectively. Vector pET-11a was obtained from Novagen (Madison, WI). *p*-Nitrophenyl phosphate (*p*NPP), phosphoserine, phosphothreonine, and phosphotyrosine were purchased from Sigma. Oligonucleotide primers were synthesized by A. Nussbaum (Harvard Medical School). Phosphotyrosyl peptide GAP^{pY460} was a gift from S. E. Shoelson

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Abbreviations: SH2, Src homology 2; PTPase, protein-tyrosine-phosphatase; PTK, protein-tyrosine kinase; *p*NPP, *p*-nitrophenyl phosphate; GF, growth factor; EGFR, epidermal GF receptor; LAR, leukocyte antigen-related.

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(Joslin Diabetes Center, Boston). All other phosphotyrosyl peptides have been previously described (13, 14).

Plasmid Construction. A 1.83-kb *Bam*HI–*Eco*RI fragment, encoding amino acid residues 54–595 of SHPTP1, was isolated from plasmid pVHC·BS (6), which contains the entire cDNA of SHPTP1 plus 5′ and 3′ untranslated sequences cloned in pBluescript II SK(+). The cDNA fragment corresponding to the N-terminal 53 amino acids was amplified by the polymerase chain reaction (PCR), using plasmid pVHC·BS as template and primers 5′-ACGTGGCATATG-GTGAGGTGGTTTCAC-3′ (27 nt) and 5′-GTACTCCAC-CAGCTCTGT-3′ (18 nt), to introduce an *Nde* I site at the translation initiation site. PCR was carried out in 10 mM Tris·HCl, pH 8.3/50 mM KCl/1.5 mM $MgCl_2$/125 μM each dNTP/10 μM each primer/2.5 units of *Taq* DNA polymerase. DNA amplification was performed for 15 cycles of denaturation (94°C; 1 min), annealing (55°C; 1 min), and extension (72°C; 1 min). The PCR product was digested with *Nde* I and *Bam*HI and the 0.17-kb fragment was purified on a 2% low melting point agarose gel. The 0.17-kb *Nde* I–*Bam*HI fragment, together with the 1.83-kb *Bam*HI–*Eco*RI fragment, was then ligated into the *Nde* I/*Eco*RI-linearized plasmid pGEX-3Xb [modified from pGEX-3X (Pharmacia) to contain an *Nde* I site in the polycloning sequence by G. Payne (Harvard Medical School)] to generate plasmid pGEX-SHPTP1. The sequence of the PCR-generated fragment was confirmed by dideoxy sequencing using the 18-nt PCR primer as the sequencing primer.

To facilitate the transfer of SHPTP1 gene between different expression vectors, the 2.0-kb *Nde* I–*Eco*RI fragment of pGEX-SHPTP1 (encoding the entire SHPTP1) was inserted into shuttle vector pCW (provided by A. Roth, University of Oregon, Eugene) to give the plasmid pHSe5-SHPTP1. In this construct, the 3′ terminus of SHPTP1 gene is flanked by a polylinker which comprises the restriction sites for *Eco*RI, *Xba* I, *Sal* I, *Pst* I, *Hin*dIII, *Cla* I, and *Bgl* II. Ligation of the 2.0-kb *Nde* I–*Cla* I fragment of pHSe5-SHPTP1 with the larger *Nde* I–*Bam*HI fragment of pET-11a (blunt-end ligation between the *Cla* I and *Bam*HI termini that have been treated with the Klenow fragment of DNA polymerase) afforded the plasmid pET-SHPTP1.

To express the PTPase domain, the 1.38-kb *Nar* I–*Hin*dIII fragment of pVHC·BS, which encodes amino acids 205–595 of SHPTP1, was inserted between the *Bam*HI and *Hin*dIII sites of plasmid pT7-7 (blunt-end ligation between the *Nar* I and *Bam*HI termini). In the resulting plasmid, pT7-PTP2, the PTPase coding sequence is fused to a sequence encoding nine amino acids (MARIRARGS) derived from the pT7-7 vector at the N terminus. Subcloning of the 1.40-kb *Nde* I–*Cla* I fragment of pT7-PTP2 in pET-11a was carried out as described above, to produce plasmid pET-PTP2.

Expression and Purification of SHPTP1 and the PTPase Domain. *E. coli* strain BL21(DE3) transformed with plasmid pET-PTP2 was grown in 1.5 liters of LB medium containing ampicillin (75 μg/ml) at 37°C to an OD_{595} of 0.6–0.7, and induced for 3 hr at 28°C with 0.4 mM isopropyl β-D-thiogalactoside (IPTG). Cells were harvested by centrifugation and resuspended in 30 ml of lysis buffer A (50 mM Tris·HCl, pH 8.0/10 mM EDTA/10 mM 2-mercaptoethanol/50 mM NaCl/1% Triton X-100) containing a protease inhibitor mixture (0.5 mM *o*-phenanthroline/0.64 mM benzamidine/0.29 mM phenylmethylsulfonyl fluoride/20 μg of soybean trypsin inhibitor per ml/20 μg of aprotinin per ml/20 μg of leupeptin per ml/20 μg of pepstatin per ml). The cells were lysed by French press and the crude lysate was centrifuged at 15,000 rpm for 15 min in a Sorvall SS-34 rotor. The clear supernatant (≈29 ml) was directly loaded onto a DEAE-Sepharose CL-6B (Sigma) column (12 × 2.5 cm), which was washed with 200 ml of buffer B (20 mM Tris·HCl, pH 8.0/10 mM 2-mercaptoethanol/10 mM NaCl/1 mM EDTA) at 2 ml/min. Fractions with significant PTPase activity were pooled (≈35 ml), diluted to 70 ml in buffer C (30 mM Mops, pH 7.2/10 mM 2-mercaptoethanol/10 mM NaCl/1 mM EDTA), and loaded onto a CM-Sepharose (Pharmacia) column (8 × 2.5 cm) equilibrated with buffer C. The column was washed with 50 ml of buffer C and then adsorbed molecules were eluted with 300 ml of buffer C plus a gradient of 10 mM to 500 mM NaCl at 1 ml/min. The active fractions (eluted at approximately 200 mM NaCl) were pooled (≈26 ml) and concentrated in a Centriprep-10 concentrator (Amicon). The resulting sample was dialyzed against buffer C and loaded onto a Pharmacia FPLC Mono S (HR 10/10) column, which was eluted with a gradient (170 ml) of 10 mM to 1 M NaCl in buffer C at a flow rate of 2 ml/min. The peak fractions (monitored by UV at 280 nm) were collected and concentrated as before to approximately 1 ml. This sample was then loaded onto a Pharmacia Superdex 75 column and eluted with buffer C plus 500 mM NaCl. The active fractions were combined and concentrated to a protein concentration of approximately 3 mg/ml. Glycerol was added to a final concentration of 33% (vol/vol) and the enzyme was stored frozen at −80°C.

The full-length SHPTP1 was purified in a similar fashion except that a 20–60% saturated ammonium sulfate precipitation of the crude lysate was included before the DEAE-Sepharose column step and the final gel filtration step was excluded from the above procedure. Active SHPTP1 was eluted off the Mono S column at approximately 400 mM NaCl and was stored frozen at −80°C in the presence of 33% glycerol.

Determination of Molecular Weights of the Recombinant PTPases. The purified full-length SHPTP1 and PTPase domain were injected onto the Superdex 75 column connected to a Pharmacia FPLC system to determine their molecular weights. Under isocratic conditions of 30 mM Mops, pH 7.2/10 mM 2-mercaptoethanol/1 mM EDTA/150 or 500 mM NaCl, bovine gamma globulin, chicken ovalbumin, and equine myoglobin eluted at 18.8 min, 23.7 min, and 29.4 min, respectively. At both NaCl concentrations, full-length SHPTP1 and the truncated PTPase domain eluted as single sharp peaks with retention times of 21.3 min and 22.6 min, respectively. These correspond to molecular weights of 72,000 and 53,000, respectively, and indicate that both molecules were eluted as monomers.

Assay for PTPase Activity. Assays for activity with *p*NPP were performed at 23°C in a 50-μl solution containing 0–80 mM *p*NPP, 100 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes) at pH 5.5 or 6.3, 10 mM dithiothreitol, 150 mM NaCl, 2 mM EDTA, and PTPase at 5–13 μg/ml. Enzyme dilutions were made in buffer C plus bovine serum albumin at 1 mg/ml. Typically, 2–5 min after the addition of enzyme, the reaction was quenched with 950 μl of 1.0 M NaOH, and the absorbance of *p*-nitrophenolate at 450 nm was determined. The nanomoles of *p*-nitrophenol released was calculated by comparison with a standard curve determined for *p*-nitrophenolate (Sigma).

To assay the dephosphorylation of phosphotyrosine or phosphotyrosine-containing peptides by the PTPases, the release of inorganic phosphate (P_i) was determined with a malachite green assay (16, 17). Assays were carried out under conditions described above (with 0–6.7 mM peptides). The reaction was quenched by the addition of 950 μl of the malachite green reagent (16), and after 10 min at room temperature, the absorbance at 660 nm was determined. The nanomoles of P_i released was calculated by comparison with a standard curve determined for P_i.

Prior to assays, all substrates were dissolved in distilled–deionized water and the pH of the resulting solution was brought to approximately 6 with HCl or NaOH.

RESULTS

Expression of Full-Length SHPTP1 and the PTPase Domain. To obtain sufficient quantities of pure SHPTP1 enzyme for enzymological and biochemical studies, we initially undertook the expression in *E. coli* of this SH2-containing PTPase as a fusion protein with maltose-binding protein (MBP). While the majority of the MBP–SHPTP1 fusion protein expressed was soluble, its specific activity with *p*NPP (at pH 5.5, 150 mM NaCl, and 10 mM *p*NPP) was only 10% of that determined subsequently for the nonfusion SHPTP1 expressed in *E. coli* (see below). The fusion protein was very susceptible to proteolysis, and our attempt to release the phosphatase from the maltose-binding protein with protease factor Xa also failed due to overwhelming nonspecific cleavage. Low PTPase activity and sensitivity to protease, taken together, may indicate that the majority of SHPTP1 in the fusion protein did not fold into a fully native structure.

We subsequently inserted the coding sequences of the full-length enzyme and the C-terminal PTPase domain (amino acids 205–595) into the *E. coli* expression vector pET-11a to generate plasmids pET-SHPTP1 and pET-PTP2, respectively (see *Materials and Methods*). In pET-PTP2, the 9-amino acid sequence MARIRARGS, derived from vector pT7-7, was added to the N terminus of the PTPase domain. Upon induction with isopropyl β-D-thiogalactoside, cells carrying either pET-SHPTP1 or pET-PTP2 overexpressed the desired proteins, which were the most prominent bands on SDS/PAGE (data not shown). Dramatically increased phosphatase activity was also easily detectable in the crude lysate of the cells transformed with the plasmids as opposed to control cells that did not harbor the plasmids. It was estimated that in both cases approximately 20% of the expressed PTPase proteins were in the soluble fraction whereas the rest was in inclusion bodies, and the active PTPases constituted 3–4% of total *E. coli* soluble protein.

The PTPase domain was purified from crude lysates by a combination of ion-exchange (DEAE-Sepharose, CM-Sepharose, and Mono S columns) and gel filtration (Superdex 75) chromatography. The purification data for a typical preparation are shown in Table 1. After gel filtration on the Superdex 75 column, the enzyme was essentially homogeneous as shown in Fig. 1 (lane 1), in which a single major band of 45 kDa is observed after SDS/PAGE and staining with Coomassie blue. The yield was typically 2.5–3.5 mg of enzyme per liter of *E. coli* cell culture.

The purification of the full-length SHPTP1 was carried out similarly but with some modifications. A 20–60% ammonium sulfate fractionation was performed with the crude lysate in attempt to remove any partially folded SHPTP1 protein. The SH2 domains of SHPTP1, when expressed in *E. coli* as glutathione-*S*-transferase fusion protein, had previously been shown to form almost exclusively inclusion bodies (G. Payne and C.T.W., unpublished results). The 20% saturated ammonium sulfate precipitation did remove some phosphatase active protein. We presume that the lessened solubility of this fraction reflects some degree of nonnative folding. After

Table 1. Purification of the PTPase domain of SHPTP1

Step	Total protein, mg*	Specific activity, μmol·min^{-1}·mg^{-1}†
Crude lysate	325	0.74
DEAE-Sepharose	120	0.90
CM-Sepharose	13	7.63
Mono S HR 10/10	6.3	15.0
Superdex 75	5.2	16.0

*From 1.5 liters of *E. coli* culture.
†The micromoles of *p*NPP (10 mM) hydrolyzed at 25°C and pH 7.2 by 1 mg of protein.

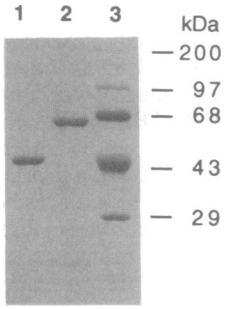

FIG. 1. Stained SDS/10% polyacrylamide gel showing the purified full-length SHPTP1 (lane 2), the truncated PTPase domain (lane 1), and molecular mass markers (lane 3).

Mono S column chromatography, 5.7 mg of SHPTP1 (from 1.5 liters of cell culture) was obtained, which showed a major band of 63 kDa on an SDS/PAGE gel (Fig. 1, lane 2).

The native molecular masses of the purified full-length SHPTP1 and the truncated PTPase domain were determined on a Superdex 75 column at pH 7.2 and in the presence of 150 mM or 500 mM NaCl. Under either set of conditions, both molecules were eluted as monomers, with apparent molecular masses of 72 and 53 kDa for SHPTP1 and the PTPase domain, respectively.

pH and Salt Dependence of the Recombinant PTPases. When assays were carried out with *p*NPP (10 mM) as substrate in a set of buffers with pH values ranging from 4.75 to 9.10 (at 150 mM NaCl), we found that the full-length SHPTP1 and the PTPase domain display distinct pH dependences in their activities (Fig. 2 *Upper*). The truncated PTPase domain is most active at pH 6.1–6.5, with a specific activity of approximately 29 μmol/min per mg of protein; the activity drops sharply as the pH becomes more acidic or basic. The full-length PTPase, however, is most active at pH 5.5–5.7 (Fig. 2 *Upper*), where the specific activity is approximately 9.5 μmol/min per mg of protein. As pH decreases from 5.5 to 4.75, or increases from 5.7 to 7.0, specific activity quickly decreases.

The ionic strength of the assay buffer can also greatly affect the activities of both enzymes (Fig. 2 *Lower*). The specific activities increase with increasing concentrations of NaCl, reach maxima at 250–300 mM NaCl, and then decrease at still higher NaCl concentrations (at pH 6.3 for the PTPase domain and pH 5.5 for SHPTP1). The activities at 300 mM NaCl are 12-fold and 7-fold higher than at 4 mM NaCl for the truncated PTPase and the full-length SHPTP1, respectively. We have also noted that the ionic strength of the assay buffer can alter the pH optima of the enzymes. For example, the pH optimum of the truncated PTPase shifted from 6.7 to 6.1 when NaCl concentration was increased from 10 mM to 300 mM (data not shown).

Catalytic Properties of the Recombinant PTPases. The catalytic activities of the recombinant PTPases toward a variety of substrates (*p*NPP, phosphotyrosine, and phosphotyrosyl peptides) were assayed at pH 6.3 (for PTPase domain) or pH 5.5 (for SHPTP1) in the presence of 150 mM NaCl (physiological NaCl concentration). Under these conditions, the PTPase domain efficiently dephosphorylated *p*NPP (V_{max} = 148 μmol/min per mg of protein for a k_{cat} = 110 sec^{-1}) and demonstrated apparent hyperbolic saturation kinetics (data not shown). However, it has a very high K_m value (38 mM), suggesting that the enzyme binds *p*NPP very weakly. Since the enzyme is extremely sensitive to the ionic strength of the assay buffer, the increasing ionic strength due to increasing substrate concentrations (0–80 mM) stimulated the enzyme activity, suggesting that the apparent K_m of 38 mM for the PTPase domain was an overestimate. We then carried out assays at 300 mM NaCl, where the further increase in ionic

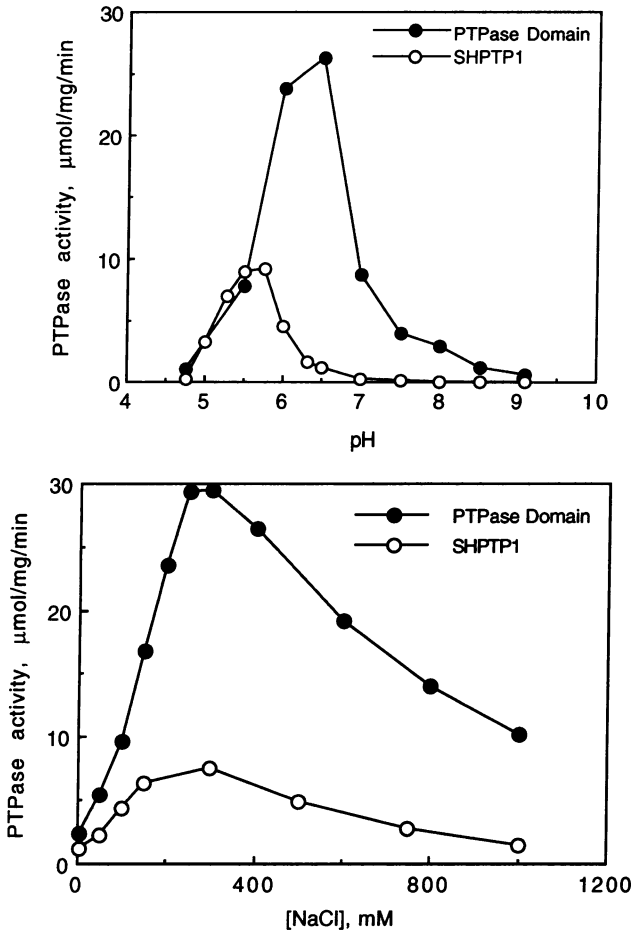

FIG. 2. (*Upper*) pH dependence of recombinant PTPases. The reactions were performed with 10 mM *p*NPP as the substrate in 50 μl containing 100 mM buffer, 10 mM dithiothreitol, 150 mM NaCl, 2 mM EDTA, and PTPase at 5–13 μg/ml. The buffers used were NaOAc/HOAc (pH 4.75), Mes (pH 5.0–6.5), imidazole (pH 7.0–7.5), and *N,N*-bis(2-hydroxyethyl)glycine (pH 8.0–9.1). (*Lower*) Effect of ionic strength on PTPase activity. Assay reactions were performed in 50 μl containing 100 mM Mes at pH 5.5 (for SHPTP1) or 6.3 (for PTPase domain), 10 mM *p*NPP, 10 mM dithiothreitol, 2 mM EDTA, PTPase at 5–13 μg/ml, and various concentrations of NaCl.

strength inhibits the enzymic activity (Fig. 2 *Lower*), and an apparent K_m of 24 mM was obtained. Therefore, the K_m of the PTPase domain for *p*NPP ranges between 24 and 38 mM, with a k_{cat}/K_m of 2.9–4.6 × 10^3 $M^{-1}\cdot sec^{-1}$. The full-length SHPTP1 also showed apparent saturation kinetics for *p*NPP, with a V_{max} of 147 μmol/min per mg of protein (k_{cat} = 155 sec^{-1}), but had a very high K_m of 148 mM and a k_{cat}/K_m of 1.0 × 10^3 $M^{-1}\cdot sec^{-1}$.

The PTPase domain showed Michaelis–Menten kinetics with phosphotyrosine, with a k_{cat} of 2.4 sec^{-1}, a K_m of 2.3 mM, and a k_{cat}/K_m of 1.0 × 10^3 $M^{-1}\cdot sec^{-1}$. These data are subject to some error (±50%) due to the presence of contaminating P_i (6.4% relative to phosphotyrosine) in the commercial phosphotyrosine sample, which complicates the malachite green assay (16, 17). Addition of 6.4% P_i to a *p*NPP sample did not significantly inhibit its hydrolysis by the PTPase, indicating that the observation that the rate of phosphotyrosine dephosphorylation is 1/50th the rate for *p*NPP is not due to product inhibition.

At substrate concentrations up to 5 mM, the PTPase domain did not result in any release of P_i from phosphoserine or phosphothreonine as determined by malachite green assay (16, 17). The enzyme also showed no detectable activity toward a ^{32}P-labeled phosphoserine (pS) peptide (DLDVPIPGRFDRRVpSVAAE) derived from the R_{II} regulatory subunit of protein kinase A (18). These results confirmed that SHPTP1 is a residue-specific PTPase (6).

The substrate specificity of the recombinant PTPases was examined with several chemically synthesized phosphotyrosyl peptides which correspond to known phosphorylation sequences of intracellular signaling molecules or the autophosphorylation sites of GF receptors. These peptides include the EGFR peptide EGFR^{pY1173}-(1167–1177) (TAENAEpYLRVA; ref. 19), the insulin receptor peptide IR^{pY1146}-(1142–1153) (TRDIpYETDYYRK; ref. 20), phospholipase C-γ peptide PLCγ^{pY771}-(766–776) (TAEPDpYGALYE; ref. 21), the T-cell receptor ζ chain peptide TCR^{pY153}-(148–157) (ATKDTpYDALH; ref. 15), p34cdc2 kinase peptide p34cdc2^{pY15}-(10–19) (IGEGTpYVVYK; ref. 22), GTPase-activating protein peptide GAP^{pY460}-(454–466) homolog (VDGKEIpYNTIRRK; ref. 23), and the C-terminal peptide of SHPTP1 itself, SHPTP1^{pY538}-(528–540) (LQSQKGQESEpYGN; ref. 6). Surprisingly, neither the full-length enzyme nor the PTPase domain showed classical saturation kinetics for any of these peptides. At peptide concentrations below 3 mM, the PTPase domain seems to behave "normally"; its specific activities toward these peptides increase either linearly or slightly hyperbolically with the substrate concentration, suggesting that the K_m values of the PTPase domain for these peptides are far greater than 3 mM. This is in contrast to other PTPases such as LAR, CD45, and human PTPase β, which have K_m values for these peptides in the low to high micromolar range (13, 14). The full-length enzyme, on the other hand, is actually stimulated by these phosphotyrosyl peptides, with specific activity increasing above linearity (data not shown). The only exception was with the p34cdc2 peptide, in which the SHPTP1 activity increases linearly with the substrate concentration. To confirm the observed phenomenon, we tested both enzymes at still higher substrate concentrations (up to 6.7 mM) with one of the peptides [GAP^{pY460}-(454–466)]. The full-length enzyme continues to show increasing activity. Strikingly, the PTPase domain also demonstrates stimulated activity at the high substrate concentration range (2–6.7 mM), although its activity seems to reach an early plateau at substrate concentration of approximately 2 mM (data not shown). A similar curve was obtained when the reactions were performed at 300 mM NaCl. We have not tested full-length phosphotyrosyl proteins as substrates.

DISCUSSION

We describe here the expression in *E. coli* of both the full-length human SHPTP1 and its catalytic domain. The ready availability of these enzymes in milligram quantities and pure form allowed us to begin to examine the catalytic properties of the enzymes. Both enzyme forms are strongly dependent on pH and ionic strength for activity, but the two have different acidic pH optima (pH 5.5 for SHPTP1 vs. pH 6.3 for the truncated PTPase domain) with *p*NPP as substrate.

In general, SHPTP1 appears to be a less efficient catalyst (k_{cat}/K_m on the order of 10^3 $M^{-1}\cdot sec^{-1}$) compared with other PTPases such as LAR, CD45, and human PTPase β, which have k_{cat}/K_m values of 10^5–10^6 $M^{-1}\cdot sec^{-1}$ (13, 14). Both the full-length and the SH2 domain-truncated form of this enzyme have very high K_m values for either artificial substrates (24–148 mM for *p*NPP) or phosphotyrosyl peptides (no saturation observed with substrate concentration up to 3 mM). None of the *in vivo* substrate(s) of SHPTP1 have been identified, and we have not yet assayed the enzyme on any full-length phosphotyrosyl protein. It is not clear what K_m or k_{cat}/K_m values will be for physiological substrates under physiological pH conditions. If they are indeed low compared with other PTPases, then posttranslational modifications (e.g., phosphorylation) and/or association with other partner

proteins might be up-regulatory. Secondary signaling molecules such as GTPase-activating protein, phospholipase C-γ, and phosphatidylinositol 3-kinase are known to associate with activated GF receptors through their SH2 domains (reviewed in ref. 1). The SH2 domains of PTPases could participate in similar regulatory mechanisms (6). When SHPTP1 associates with other phosphotyrosyl proteins, this might bring substrates (e.g., other phosphotyrosyl residues on the protein) to the close vicinity of the PTPase domain, thus increasing the effective concentration of the substrate and allowing the dephosphorylation event to occur. The high K_m values of this enzyme for all tested phosphotyrosyl peptides also suggest that this enzyme might be rather nonspecific, although it remains to be determined what affinity and k_{cat} it may have for specific partner phosphotyrosyl protein substrates. In this case, the specificity of the phosphatase might be dictated by the precomplexation specificity of the SH2 domains. Preliminary experiments indicate that a homologous SH2-containing PTPase, SHPTP2, associates with EGFR, platelet-derived GF receptor (PDGFR), and insulin receptor substrate 1 (IRS-1) (R. J. Lechleider, R. Freeman, and B.G.N., unpublished results).

The activity of the full-length PTPase is stimulated by phosphotyrosyl peptides but not *p*NPP. Initially, we thought that the SH2 domains of the PTPase might be binding to its own C-terminal domain and, as in proposals for Src-like kinases (1), physically block the PTPase active site from substrates. Phosphotyrosyl peptides would then compete with the SH2 domains for binding their own C terminus and render the PTPase active site accessible to substrates. However, when we examined the truncated PTPase, which lacks the SH2 domains, at sufficiently high phosphotyrosyl peptide concentrations (>3 mM) the same type of stimulation was also observed. Thus, the activity stimulation is clearly not a result of interactions between the SH2 domains and the C terminus. This is not surprising, since the protein produced in *E. coli* is presumably free of tyrosine phosphorylation. Of course, this has not ruled out the possibility that such an interaction could occur in eukaryotic cells if one or more of the three tyrosine residues in the C terminus undergo phosphorylation (6). In fact, *in vivo*, SHPTP1 is phosphorylated on serine residues and appears to be phosphorylated on tyrosine under some conditions (U. Lorenz and B.G.N., unpublished results). It will be of interest to determine the relationship between the PTPase activity and its phosphorylation state. As to the mechanism of stimulation of SHPTP1 activity, one hypothesis would be that there might exist another binding site for phosphotyrosyl peptides in the PTPase domain, and binding by phosphotyrosyl peptides to this site would induce a favorable conformational change of the PTPase. Another possibility is that the hydrophilic C terminus may modulate PTPase activity as an autoinhibitor, shielding the catalytic center from substrates as described for myosin light chain kinase (24), protein kinase C (25), and calcineurin (26). Autoregulation of activity by the C terminus has also been reported for a human T-cell PTPase (27). It is also possible that the PTPase expressed in *E. coli* is not completely folded into the native structure.

We thank Drs. G. Payne and S. Tabor for expression vectors and Dr. S. Shoelson for the phosphotyrosyl peptide GAP^{pY460}-(454–466) homolog. We acknowledge Dr. L. Stolz for testing the PTPase on the phosphoserine peptide and Dr. A. Nussbaum for synthesis of oligonucleotide primers. Drs. H. Cho, A. Kuliopulous, and H. Saito are thanked for their helpful suggestions and critical reading of this manuscript. This work is supported by National Institutes of Health Grant GM20011. D.P. is supported by a Damon Runyon–Walter Winchell Cancer Research Fund Fellowship, DRG-1172.

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