The Properties of the Protein Tyrosine Phosphatase PTPMEG*

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We previously cloned a cDNA encoding a protein tyrosine phosphatase (PTP) containing sequence homology to protein 4.1, designated PTPMEG. Recombinant protein and amino- and carboxyl-terminal peptides were used to obtain polyclonal antibodies against PTPMEG to identify endogenous PTPMEG in A172 cells and to show that the enzyme is primarily localized to the membrane and cytoskeletal fractions of these cells. We prepared recombinant protein in SF9 and COS-7 cells to further characterize it. The protein was phosphorylated in both cell types on serine and threonine residues. The multiple sites of phosphorylation were all within the intermediate domain of the protein between amino acids 386 and 503. This region also contains two PEST sequences and two proline-rich motifs that may confer binding to Src homology 3 domains. The recombinant protein was cleaved by trypsin and calpain in this region and thereby activated 4–8-fold as assayed using Raytide as substrate. We immunoprecipitated the protein from human platelets with both amino- and carboxyl-terminal antipeptide antibodies to assess the state of the enzyme in these cells. The full-length molecule was found in extracts from unstimulated platelets, whereas extracts from both calcium ionophore- and thrombin-treated platelets contained proteolyzed and activated forms of the enzyme, indicating that proteolysis by calpain is evoked in response to thrombin. Prior incubation of platelets with calpeptin, an inhibitor of calpain, blocked the agonist-induced proteolysis.

Protein tyrosine phosphorylation regulates fundamental cellular events, including passage through the cell cycle, proliferation, differentiation, and cytoskeletal organization. This process is balanced by protein tyrosine kinases and protein tyrosine phosphatases (PTPs)1 (1). In most cases protein tyrosine kinase action stimulates processes, whereas PTPs inhibit them, although there are several situations in which the converse is true. (2–5). There is much greater PTP activity than protein tyrosine kinases, which may account for the facts that protein tyrosine phosphorylation in response to agonists is transient and the levels of protein tyrosine phosphate in unstimulated cells are quite low.

There are more than 40 PTPs in mammalian cells that share 30–50% amino acid identity in a 300-residue catalytic motif (6, 7). Most of these enzymes cleave phosphate from many substrates and thus lack substrate specificity toward artificial substrates in vitro (8–10). These findings have led to the speculation that the specificity and activity of PTP are determined by structures located outside the catalytic domain where the individual PTPs differ. It has been proposed that the sequences outside the catalytic motifs serve as “zip codes” to localize particular PTPs to distinct cellular compartments that thereby limit access to substrates. These noncatalytic domains also control enzyme activity by protein modifications, including changes in phosphorylation, proteolytic cleavage, or protein-protein interactions, and restrict or enhance accessibility to substrates (11).

We have cloned a cDNA from a megakaryocyte cDNA library encoding a 926-amino acid cytosolic protein tyrosine phosphatase that contains several possible regulatory domains outside the catalytic domain (12). The amino-terminal 367 amino acids are homologous to erythrocyte cytoskeleton protein 4.1. A central domain contains two PEST sequences that predict rapid proteolysis and a 100-amino acid motif designated GLGF or DHR that is found in a variety of other proteins as described below. The catalytic domain is at the carboxyl terminus of the protein. There is a family of proteins that share homologous 4.1 domains including the cytoskeletal proteins 4.1, ezrin, moesin, radixin, and merlin. The family also includes the protein tyrosine phosphatases PTPH1 (13), PTPBAS (14), and PTPD1 (15). These proteins link to the plasma membrane by binding to integral membrane proteins and to cytoskeletal elements by binding to other proteins. Erythrocyte protein 4.1 binds to transmembrane band 3 and glycophorins A and C (16, 17). Ezrin, radixin, and moesin bind to the transmembrane protein CD44 (18). Protein 4.1 also forms complexes with two membrane-associated guanylate kinase family members, p55 and hdlg (homologue of Drosophila disc large tumor suppressor protein) (19, 20). These proteins have a DHR motif at the amino terminus, followed by an SH3 domain and a guanylate kinase homology domain (21, 22). There are 27 proteins reported to contain DHR motifs, including PTPMEG, PTPH1, and PTPBAS (23). Although the function of this motif is not known, the proteins that contain it are located in the membrane or cytoskeleton, and it is proposed to mediate binding to 4.1 domain-containing proteins (21–23). The cellular location of the 4.1- and DHR-containing PTP is unknown, although a membrane or cytoskeletal location seems likely based on the data from homologous proteins.

We have now identified endogenous PTPMEG in cultured A172 glioblastoma cells and have shown that the protein is mainly associated with membranes and cytoskeleton. We also found that the protein is phosphorylated on both serine and threonine at multiple sites in vivo and in vitro. The major site of phosphorylation is in the putative SH3 binding domain that is between the 4.1 domain and the PEST domain. PTPMEG is cleaved in vitro by both trypsin and calpain and is thereby

1 The abbreviations used are: PTP, protein tyrosine phosphatase; CHAPS, 3-[3-cholamidopropyl]dimethylammoniomethyl-1-propanesulfonic acid; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; SH, Src homology; DHR, discs-large homologous region; Ni2+NTA, nickel-nitrilotriacetic acid.

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activated. The cleavage site of trypsin is in the middle of the molecule. We have also shown proteolysis and activation of PTPMEG in intact platelets in response to calcium ionophore and thrombin stimulation.

EXPERIMENTAL PROCEDURES

Preparation of Antibodies to PTPMEG—Peptides were synthesized corresponding to the amino- and carboxyl-terminal regions of PTPMEG (12). The amino-terminal peptide is MTSRFRPAGRTC. The carboxy-terminal peptide is CEGFVKPLTTSTNK. The cysteine residues are not present in the PTPMEG sequence and were added to facilitate coupling to keyhole limpet hemocyanin. The peptides were conjugated to keyhole limpet hemocyanin (Sigma) carrier protein following the manufacturer’s directions (QiAexpress purification kit; Qiagen). Both antipeptide antibodies and those against full-length recombinant PTPMEG were assayed in 1 ml of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM dithiothreitol, 1 mM EDTA, and protease inhibitors (lysis buffer) was tested by immunoblotting and by immunoprecipitation of both recombinant and native PTPMEG protein from Sf9 and A172 cells, respectively. Antipeptide antibodies were affinity purified as described above. 20 mg of each peptide was coupled to 2 ml of SulfoLink coupling gel (Pierce) following the manufacturer’s directions. Rabbit antiserum was diluted with 1 vol. of phosphate-buffered saline containing 157 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, and 1.4 mM KH2PO4, and mixed for 30 min at 4°C. The antibodies were then eluted with 0.1 mM glycine, pH 2.5, and immediately neutralized with 1 mM Tris-HCl, pH 9.0.

Immune Complex Protein Tyrosine Phosphatase Assay—Cell lysates were prepared in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100 or Nonidet P-40, 1 mM diithiotreitol, 1 mM EDTA, and protease inhibitors, including 2 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.5 μg/ml pepstatin, and 0.2 mM phenylmethylsulfonyl fluoride. Affinity-purified antipeptide antibody (5 μg) was mixed with each sample. After 1 h at 4°C, 30 μl of a 50% slurry of protein A-Sepharose was added, and samples were mixed by rolling at 4°C for 1 h. The protein A-Sepharose was then pelleted and washed three times with the same buffer. Enzyme activity was measured by suspending the protein A-Sepharose in 40 μl of 10 mM imidazole-HCl (pH 7.2) containing 1 mg/ml bovine serum albumin and 14 mM d-mercaptoethanol. This mixture was then incubated at 30°C for 5 min, followed by the addition of 20,000 cpm of [32P]Raytide substrate (1000 cpm/pmol). After an additional 5–10 min incubation at 30°C, the reaction was stopped by the addition of 10 μl of charcoal mixture (25). The charcoal was pelleted by centrifugation, and the supernatant was used for immunoprecipitation with affinity-purified amino- or carboxyl-terminal antipeptide antibodies followed by immune complex PTPase assay as described above.

Phosphoamino Acid Assay and Phosphopeptide Analysis—Phosphoamino acid analysis and phosphopeptide analyses were performed using a Hunter thin layer plate electrophoresis system (C.B.S. Scientific Co.). The manufacturer’s protocol was followed to prepare samples.

Baculovirus Expression of PTPMEG and Truncated Forms of PTPMEG—The 5′- and 3′-untranslated regions of PTPMEG cDNA were deleted separately by a PCR strategy using in which a unique EcoRV site was added to the 5′-end, and an EcoRV site was added to the 3′-end. The 5′- and 3′-end untranslated region-deleted PCR product was ligated into a Bluescript vector (Stratagene) at the EcoRV site to create PTPMEGor in which base pair 1 corresponds to A of the initiator methionine of PTPMEG (12). The active site cysteine of PTPMEG was mutated to serine by PCR to generate the mutant cDNA PTPMEGCS. All possible mutations were sequenced by PCR to ensure that no spurious mutations were generated. PTPMEGCS cDNA was placed in pVL1393 baculovirus transfer vector (Pharmingen) followed by cDNA for 7 histidines and a stop codon to generate pPTMPEG-H.

Truncated forms of PTPMEG were also expressed in baculovirus. The catalytic domain was deleted from pPTMPEG-H to generate pPTMPEG1–647 encoding amino acids 1–647. PCR was used to create a construct harboring the 4.1 homology domain (amino acids 1–367) by a PCR strategy using PTPMEGCS as a template to create pPTMPEG1–647. A PCR strategy was also used to create a construct harboring the intermediate domain using pPTMPEG1–647 as template. pPTMPEG-G2138–647 encodes the intermediate domain minus amino acids from E229 to Q300 that were lost, since they were not in the template.

The transfer vectors containing the various constructs were co-transfected into S9 insect cells following the manufacturer’s protocol (Pharmingen). Recombinant viruses were plaque purified and amplified. Phosphorylation of recombinant proteins was evaluated by plating 3 × 106 S9 cells in six-well dishes and infecting with recombinant baculovirus at a multiplicity of infection of about 1. After 48 h, cells were washed and incubated in phosphate-free medium with 0.5 mM calcium. After 12 h the cells were washed from the plates; washed with calcium-buffered saline, and then lysed in 0.5 ml of buffer containing 20 mM Tris, pH 7.9, 0.5% Triton X-100, 10 mM imidazole, 0.5 mM NaCl, 1 mM sodium orthovanadate, 50 mM NaF, and protease inhibitors (lysis buffer). Recombinant proteins were purified on Ni2+–NTA resin. Samples of eluates (10 μl) were separated on 10% SDS-PAGE and transferred to nitrocellulose membranes. After autoradiography, membranes were immunoblotted with rabbit antisera against PTPMEG.

Stable Expression of PTPMEG and Active Site Mutant PTPMEG in COS-7 Cells—Both PTPMEGOR (native) and PTPMEGCS (active site mutant) were excised from Bluescript plasmid by EcoRV digestion and_
inserted into the blunted EcoRI site in a pCEN vector to create pPTPMEGORF and pPTPMEGCS. The pCEN vector was a gift from Dr. John Majors (Washington University). It is a Bluescript vector containing a cytomegalovirus promoter and following the cloning site has an encephalomyocarditis virus internal ribosomal entry site followed by a neomycin resistance gene, thereby allowing for the translation of a polycystronic mRNA encoding the gene of interest and conferring neomycin resistance. Stable cell lines expressing these constructs were obtained by neomycin selection of clones that were subsequently screened for expression of recombinant proteins by immunoblotting.

RESULTS

Identification of Endogenous PTPMEG—In initial experiments we carried out Northern blot analysis using a commercial multitissue Northern blot and found that PTPMEG was expressed in most tissues, most highly in skeletal muscle and brain (data not shown). We also did Northern blot analysis on 11 different cultured cell lines from a variety of tissues as described previously and found that PTPMEG was most highly expressed in A172 cells, a human glioblastoma cell line (28).

PTPMEG protein was identified using rabbit polyclonal antibodies to both synthetic amino- and carboxyl-terminal peptides and to the recombinant full-length protein expressed in Sf9 cells. These antibodies recognize PTPMEG in immunoblots and also immunoprecipitate the protein, as shown in Fig. 1. In this experiment affinity-purified anti-amino-terminal peptide antibody immunoprecipitated a 116-kDa protein from both Sf9 and A172 cells that was recognized by anti-PTPMEG antibody in the immunoblot shown. The immunoblottting band co-migrates with a Coomassie Blue-staining band of PTPMEG purified to homogeneity from Sf9 cells (data not shown). We also measured protein tyrosine phosphatase activity in immunoprecipitates from A172 cells, as shown in the time and concentration curves in Fig. 2. There was no 116-kDa protein or protein tyrosine phosphatase activity in immunoprecipitates made using non-immune rabbit serum. These antibodies also immunoprecipitated a 116-kDa protein and protein tyrosine phosphatase activity from mouse brain (data not shown). We conclude that the 116-kDa protein and the enzyme activity we measured are specific to PTPMEG.

Subcellular Localization of PTPMEG—We used A172 cells to determine the subcellular localization of PTPMEG. Initially, cells were separated into soluble, membrane, and cytoskeletal fractions, as shown in Fig. 3A. The same proportion of each fraction was used for immunoprecipitation and the subsequent immunoblot. PTPMEG (Fig. 3A, arrow) was found mainly in the membrane and cytoskeletal fractions, consistent with its containing a 4.1 domain. We also measured protein tyrosine phosphatase activity in immunoprecipitates of these fractions, as shown in Fig. 3B. 50% of PTPMEG protein and activity was found in the detergent-soluble membrane fraction, 30% in the detergent-soluble cytoskeleton fraction, and 20% in the cytosol fraction. This experiment has been repeated three times with the same results. We also fractionated A172 cells in another way, as described under “Experimental Procedures,” to show that PTPMEG is associated with plasma membranes, as shown in Fig. 3C. An equal proportion of each fraction was immunoprecipitated and immunoblotted. PTPMEG protein was only found at the interface between the 1.2 and 2 M sucrose layers, which contains mainly low density membranes, including plasma membranes and golgi. Note that no PTPMEG was found in the cytosol fraction using this method of cell lysis, which is gentler than that used in Fig. 3A (hypotonic lysis versus sonication).
Phosphorylation of PTPMEG—Since many protein tyrosine phosphatases are regulated by phosphorylation (29–34), we studied the state of phosphorylation of PTPMEG in Sf9 cells expressing recombinant protein. We labeled Sf9 cells infected with recombinant baculovirus harboring pPTPMEG-H with $^{32}$PO$_4$. Cell lysates were then immunoprecipitated with affinity-purified antipeptide antibody and immunoblotted with PTPMEG antiserum. C, cytosol fraction; M, soluble membrane fraction; CS, soluble cytoskeletal fraction. Arrow. PTPMEG. B, one-tenth of the total volume was used for immune complex tyrosine phosphatase assay. The activity is shown as the percentage of the total. C, A172 cells from two 150-mm dishes were homogenized and adjusted to contain 0.25 M sucrose, nuclei were separated, and the extract was further fractionated with a step sucrose gradient consisting of 2.0 M (bottom) and 1.2 M (top) sucrose as described (26). Equal volumes of each fraction were immunoprecipitated and immunobotted as described above. Lane C, recombinant PTPMEG; lane 1, cytosol fraction; lane 2, material at the interface between 0.25 and 1.2 M sucrose; lane 3, material at the interface between 1.2 and 2 M sucrose; lane 4, 1% Triton X-100 soluble nuclear fraction.

We expressed deletion constructs of PTPMEG that harbored a 7-histidine residue tag at the carboxyl terminus in Sf9 cells to further localize the sites phosphorylated. These included pPTPMEG1–647H, in which the catalytic domain was deleted, pPTPMEG1–347H, encoding the 4.1 domain, and p368–647H, which encodes the intermediate domain. The catalytic domain deletion mutant was phosphorylated as well as the full-length protein, suggesting that none of the sites of phosphorylation were within the catalytic domain, as shown in Fig. 5C. The construct expressing the 4.1 domain was not phosphorylated, indicating that the sites are not within this region either. The intermediate domain construct was phosphorylated as well as the full-length protein, with a phosphopeptide map that was very similar to that of the full-length protein (Fig. 5B, arrowheads indicate common peptides), indicating that the sites of phosphorylation are within this region. We also fractionated radiolabeled tryptic peptides of full-length PTPMEG by micro-
bore high performance liquid chromatography and found five major peptides that contained radioactivity. One major peptide was sequenced and found to correspond to amino acids 386–400 (NSISDDRLETQSLPSRSPPGTPNGR). Other radiolabeled tryptic peptides sequenced began at amino acids 413 and 473. All of these peptides are within the intermediate domain and contain multiple serine and threonine residues that are candidates for phosphorylation.

The stoichiometry of PTPMEG phosphorylation in Sf9 cells was 4–6 mol of phosphate/mol of protein.

We also labeled A172 cells with $^{32}$PO$_4$ to establish that endogenous PTPMEG was also phosphorylated. Labeled PTPMEG was identified after immunoprecipitation, SDS-PAGE, transfer to a nitrocellulose membrane, immunoblotting, and autoradiography (data not shown).

**FIG. 4.** Phosphorylation of PTPMEG in vitro. A, Sf9 cells infected with baculovirus-expressing PTPMEG ORF for 2 days were labeled with 0.5 mCi/ml of $^{32}$PO$_4$ for 12 h. Uninfected Sf9 cells were also labeled. PTPMEG was then immunoprecipitated using the amino-terminal antipeptide antibody and separated by 10% SDS-PAGE and transferred to nitrocellulose. After autoradiography (right) the membrane was immunoblotted with the anti carboxyl-terminal peptide antibody (left). Lane 1, uninfected Sf9 cells; lane 2, Sf9 cells expressing PTPMEG. B, phosphorylation in COS-7 cells overexpressing PTPMEG. One confluent 100-mm dish of COS-7 cells expressing native PTPMEG (clone N30) was labeled with 1 mCi/ml $^{32}$PO$_4$ for 4 h. The membrane fraction was then separated, and protein was solubilized in 1% Triton X-100. Equal amounts of lysate were then immunoprecipitated using affinity-purified anti-amino-terminal peptide antibody (N1N), anti carboxyl-terminal peptide antibody (N1C), and control (IgG). The immune complexes were separated by 8% SDS-PAGE. The autoradiograph is shown on the right, and the immunoblot with anti-PTPMEG antibody is shown on the left.

Proteolysis of PTPMEG—Deletion of the carboxyl-terminal 11 kDa of PTP1B in vitro activates the enzyme (35). This same region of PTP1B is cleaved by calpain on platelet activation (36), implying that enzyme activity is thus regulated in vitro. We therefore studied the effect of proteolysis on PTPMEG. Trypsin treatment of recombinant PTPMEG purified from Sf9 cells activated the enzyme, as shown in Fig. 6A. Trypsin similarly activated endogenous PTPMEG from A172 cells, as shown in Fig. 6B. To delineate the cleavage site, recombinant PTPMEG with a histidine tag was first bound to Ni$^{2+}$-NTA resin via its carboxyl-terminal tag. The protein bound to the resin was then treated with various amounts of trypsin, and the resin was washed to elute cleaved peptides. A sample of the resin was assayed for tyrosine phosphatase activity, as shown in Fig. 7C. There was a 7-fold increase in enzyme activity bound to the resin. The peptides bound to the resin were also eluted and separated by SDS-PAGE. The proteolytic peptides were then visualized by immunoblotting with the carboxyl-terminal peptide antibody, the amino-terminal peptide antibody, and the antibody against the full PTPMEG. The pattern shown by immunoblotting with PTPMEG antibody is shown in Fig. 7A; that obtained by blotting the same membrane with the carboxyl-terminal peptide antibody is shown in Fig. 7B. The increased tyrosine phosphatase activity correlated with the appearance of a 55-kDa peptide (Fig. 7, arrowheads). The peptides between 30 and 50 kDa seen in Fig. 7A were also recognized by the amino-terminal peptide antibody (data not shown), indicating that these are amino-terminal sequences that remain associ-
ated on the resin after proteolysis. This experiment demonstrates that the activated form is the carboxyl-terminal 55 kDa of the protein and that the cleavage that leads to activation of the enzyme is located within the intermediate domain of the molecule. Since a putative PEST sequence is found from amino acids 436–461, the cleavage site is most likely in that area. Furthermore, the product of cleavage in this region would be about 55 kDa. We also studied the effect of the protease calpain on PTPMEG. Calpain is a cytosolic cysteine protease and has been implicated in a variety of processes (37–39). Calpain also activated PTPMEG in a time- and concentration-dependent manner, as shown in Fig. 8.

To determine whether proteolysis occurs in intact cells, we carried out experiments wherein homogenates of human platelets were immunoprecipitated with amino- and carboxyl-terminal antipeptide antibodies, and tyrosine phosphatase activity was assayed. The activity precipitated from homogenates of unstimulated platelets was similar using the amino- and carboxyl-terminal antibodies, indicating that platelets contain intact PTPMEG, as shown in Fig. 9. When platelets were stimulated with the calcium ionophore A23187 in the presence of calcium ions, there was a rapid loss of amino-terminal precipitable enzyme, indicating proteolysis. There was also concomitant increased enzyme activity precipitated by the carboxyl-terminal antibody, indicating activation of the enzyme, as shown in Fig. 9A. At later times the activity decreased, possibly due to further inactivating proteolysis. There was no proteolysis apparent when platelets were preincubated with calpeptin, indicating the proteolysis was mediated by calpain. We repeated this experiment using thrombin to determine whether a physiological platelet agonist would also induce calpain mediated proteolysis and activation, as shown in Fig. 9B. Thrombin gave a similar result, although the proteolysis appears not to be total in this case, since a small amount of amino-terminal antibody-precipitable enzyme remains. This proteolysis was also inhibited by calpeptin preincubation. This result implies that PTPMEG is proteolytically processed and activated in vivo.

We used the same protocol to determine whether PTPMEG was proteolyzed in A172 cells. Homogenates of these cells were immunoprecipitated with amino- and carboxyl-terminal antibodies and assayed for protein tyrosine phosphatase activity. We consistently found 20% greater enzyme activity precipi-
tated by carboxyl-terminal antibodies, all of which was found in the cytosol fraction. A12387 treatment of cells did not alter this result. Thus we do not know whether under some conditions there is “physiological” proteolysis in these cells. We could not.

**DISCUSSION**

A summary of the structure of PTPMEG is shown in Fig. 10. In the current study we have defined several features of the intermediate domain. Several sites of serine and threonine phosphorylation are located in this region. There is cleavage by trypsin in this region that leads to activation of the tyrosine phosphatase. Two PEST sequences were found in this region from amino acids 436–461 (score of 9.3) and 490–503 (score of 1.9) (40). There are also two proline-rich sequences at amino acids 389–397 and 462–468 that could bind to SH3 domains of some signaling molecules (41, 42). A DHR motif was found in the carboxyl-terminal part of this domain from amino acids 518–599 (23).

A potential localization for PTPMEG is suggested by its homology to the amino-terminal 30 kDa of protein 4.1 and its possession of a DHR motif. Protein 4.1 acts as a linker protein that connects the cytoskeleton to the membrane in erythrocytes (for review, see Ref. 43). The amino-terminal domain binds to the membrane protein glycoporphin, and its carboxyl-terminal region binds to actin and spectrin filaments. Several other proteins that contain a region homologous to the amino-terminal protein 4.1 domain are also localized to membranes. Ezrin binds to the inner face of fibroblast membranes (44). Ezrin, moesin, and radixin associate with an integral membrane protein, CD44 (18). The DHR motif has been found in numerous proteins, most of which are localized to membranes, cytoskeleton, or adhesion junctions (23). The DHR motif is found in the homologous protein tyrosine phosphatases PTPH1 and PTP-BAS, although the cellular location of these proteins in un-
known. PTPBAS is presumed to localize to membranes, since it forms complexes with the membrane receptor Fas (45). We now report that PTPMEG is localized to membrane and cytoskeletal fractions in COS-7 cells expressing the recombinant protein\textsuperscript{2} as well as in A172 cells, in which the endogenous protein is similarly located. The existence of noncatalytic motifs such as 4.1, DHR, and the proline-rich sequences that may bind to SH3 domains may serve as zip codes to localize the protein as well as to provide sites for other protein-protein interactions, including binding to potential substrates (11).

We found that the intermediate domain is highly phosphorylated on serine and threonine but not tyrosine in both S9 and COS-7 cells. We have no evidence that these phosphorylations affect enzyme activity, since phosphorylation of recombinant protein \textit{in vitro} did not affect activity toward Raytide. We do not have forms of PTPMEG with varying states of phosphorylation and thus cannot evaluate the affect of phosphorylation on activity, proteolysis, or reactivity with various antibodies. PTPH1, the most closely related protein tyrosine phosphatase to PTPMEG, is also phosphorylated on serine and threonine with little change in activity (46). Serine phosphorylation of PTP1B varies during the cell cycle, although its subcellular distribution and activity toward artificial substrates is unchanged (30). The phosphorylation of PTP-PEST by protein kinase C \textit{in vitro} or in cells stimulated by phorbol esters decreases tyrosine phosphatase activity (31). Tyrosine phosphorylation of PTP1D in cells overexpressing platelet-derived growth factor B enhances the catalytic activity (29) and recruits Grb2 (47).

Possibly these phosphorylations serve to mediate binding to other proteins or activity toward specific substrates. Protein 4.1 is phosphorylated on serine and tyrosine \textit{in vivo} (43), and these phosphorylations regulate the interactions of this protein with spectrin, actin, and band 3.

The activation of PTPMEG by proteolysis is striking but of uncertain physiological function. It is possible that the 4.1 domain both anchors the protein to the membrane and inhibits it. There is accumulating evidence that intracellular signals may be influenced by proteolysis (37–39). Proteolytic activation of PTP1B occurs after cleavage of the carboxyl-terminal 11-kDa peptide (35). PTP1B undergoes proteolysis in response to platelet activation. Calpain is responsible for the cleavage and cleaves the carboxyl-terminal sequence, which results in activation of the enzyme and translocation from membrane to cytosol (36). Proteolytic cleavage of the carboxyl-terminal 4 kDa of SHPTP2 results in a 27-fold increase in activity (48). Deletion of the SH2 domain also enhances the activity 12–45-fold (49). It has been proposed that the SH2 domain inhibits the enzyme in the absence of substrate (48). Calpain is a calcium-dependent cytosolic protease that becomes associated with the plasma membrane on stimulation (38). In addition to protein kinase C, several high molecular weight cytoskeletal proteins, including actin-binding protein, spectrin, talin, and myosin heavy chain, have been found as the targets of calpain \textit{in vivo} (50, 51). Activation of calpain has been shown to cause cytoskeletal rearrangement in aggregating platelets (52). We have shown that calpain is able to cleave and activate PTPMEG \textit{in vitro} and \textit{in vivo}. This occurs in intact platelets in response to calcium ionophore and the physiological agonist thrombin.

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