Identification of Phosphocaveolin-1 as a Novel Protein Tyrosine Phosphatase 1B Substrate†

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ABSTRACT: Protein tyrosine phosphatase 1B (PTP1B) is implicated in a number of signaling pathways including those mediated by insulin, epidermal growth factor (EGF), and the Src family kinases. The scaffolding protein caveolin-1 is also a participant in these pathways and is specifically phosphorylated on tyrosine 14, when these pathways are activated. Here, we provide evidence that PTP1B can efficiently catalyze the removal of the phosphoryl group from phosphocaveolin-1. Overexpression of PTP1B decreases tyrosine 14 phosphorylation in caveolin-1, while expression of the substrate-trapping mutant PTP1B/D181A causes the accumulation of phosphocaveolin-1 and prevents its dephosphorylation by endogenous PTPs. We further demonstrate that PTP1B physically associates with caveolin-1. Finally, we show that inhibition of PTP1B activity with a potent and specific small molecule PTP1B inhibitor blocks the PTP1B-catalyzed caveolin-1 dephosphorylation both in vitro and in vivo. Taken together, the results strongly suggest that caveolin-1 is a specific substrate for PTP1B. Identification of caveolin-1 as a PTP1B substrate represents an important new step in further understanding the signaling pathways regulated by PTP1B.

Protein tyrosine phosphorylation controls the activity of many signaling proteins and is involved in almost all eukaryotic cell activities. The state of tyrosine phosphorylation undergoes dynamic and reciprocal regulation by two large enzyme families: protein tyrosine kinases and protein tyrosine phosphatases (PTPs).[1] By adding or removing phosphate groups to or from substrate proteins, protein tyrosine kinases and PTPs play crucial roles in cellular proliferation, differentiation, metabolism, and survival (*1*, *2*). As important as the tyrosine kinases, the PTPs have been implicated in a number of diseases including cancer, diabetes, and inflammation (*3*). For example, genetic and biochemical evidence indicates that PTP1B is an important negative regulator of insulin- and leptin-mediated pathways (*4*). In particular, mice lacking PTP1B are hyperresponsive to insulin and resistant to diet-induced obesity (*5*, *6*), implicating PTP1B as a key therapeutic target in the treatment of type 2 diabetes and obesity.

However, besides a role in insulin and leptin signaling, PTP1B may also associate with several other physiological and pathological processes, including the suppression of cell transformation by the *Neu* oncogene (*7*, *8*) and v-Src (*9*), activation of the Src kinase (*10*−*12*), and antagonizing signaling by the EGF receptor (*13*−*16*) and the oncoprotein $p210^{bcr-abl}$ (*17*). Taken together, these results suggest that PTP1B may be a participant in several signaling pathways. Because PTP1B may be a regulator of multiple signaling pathways and it can both enhance and antagonize a cellular event, the physiological relevance of PTP1B in these processes needs to be established. This is an important prerequisite for the development of PTP1B-based therapeutics for type 2 diabetes and obesity.

Despite extensive studies, the unique functional contributions made by PTP1B to individual signal pathways have not yet been fully defined. In part, this is due to a lack of full knowledge of the physiological substrates of PTP1B. Our hypothesis is that PTP1B plays diverse roles in signal transduction because it recognizes distinct substrates in different cellular contexts. Consequently, the identification of PTP1B substrates is an essential step toward a complete understanding of the physiological function of this enzyme. The yeast two-hybrid approach in general has proven ineffective for PTP substrate identification because of the requirement for tyrosine phosphorylation (there are no protein tyrosine kinases in yeast). An effective strategy to identify and characterize PTP substrates has been to employ PTP

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[1] Abbreviations: PTP, protein tyrosine phosphatase; CSK, C-terminal Src kinase; GST, glutathione *S*-transferase; *p*NPP, *p*-nitrophenyl phosphate; EGF, epidermal growth factor; IR, insulin receptor.

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substrate-trapping mutants, which retain the affinity for substrates but are unable to catalyze substrate dephosphorylation (*14*, *15*, *18*). Although a number of substrates have now been identified for PTP1B, the majority of them are tyrosine kinases themselves, which usually initiate a signaling cascade. As such, it is not clear whether there are other downstream targets of PTP1B.

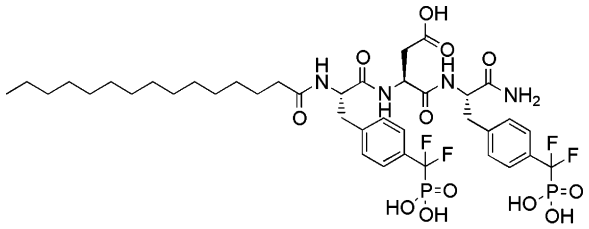
Caveolin-1 is a small integral membrane protein and the principal component of caveolae membrane microdomains within the cell (*19*, *20*). Caveolae are 50−100 nm flask-shaped invaginations at the plasma membrane that are mostly found in terminally differentiated cells such as fibroblasts, endothelial cell, adipocytes, and skeletal muscle cells (*21*, *22*). Caveolae have been shown in recent years to function as the sites of important dynamic regulatory events in signal transmission (*19*). Many signaling molecules, including heterotrimeric G proteins, Src family tyrosine kinases, receptor protein tyrosine kinases, Ras-related GTPases, endothelial nitric oxide synthase, and protein kinase C, are concentrated within caveolae, via protein−protein interactions with a 20 amino acid scaffolding domain in caveolin-1 (*23*, *24*).

In addition to serving as a scaffolding protein for signaling molecules, caveolin-1 is also directly involved in signaling cascades as a substrate of tyrosine kinases. Historically, caveolin-1 was first identified as a major tyrosine-phosphorylated protein in fibroblasts transformed by Rous sarcoma virus (RSV), which encodes the avian cancer-causing oncogene v-Src (*25*, *26*). Microsequence analysis, deletion, and site-directed mutagenesis revealed that this phosphorylation event occurs at tyrosine 14 in caceolin-1 (*27*). Subsequently, Tyr14 in caveolin-1 was found to be constitutively phosphorylated in Src- and Abl-transformed cells and transiently phosphorylated in a regulated fashion, upon epidermal growth factor (EGF) or insulin stimulation (*27*−*31*). Given the dynamic nature of protein tyrosine phosphorylation in the cell, the level of Tyr14 phosphorylation must be controlled by both protein tyrosine kinases and the PTPs.

The low molecular weight PTP was shown previously to be able to dephosphorylate phosphocaveolin-1 (*32*). However, the identities of the classical PTPs that specifically catalyze the dephosphorylation of Tyr14 of caveolin-1 have remained elusive. Using a combination of direct biochemical analysis, substrate-trapping, and a potent selective small molecule inhibitor, we provide strong evidence that phosphocaveolin-1 is a novel PTP1B substrate.

EXPERIMENTAL PROCEDURES

Materials. The cDNA encoding caveolin-1 was subcloned into the CMV-based vector pCB7, as described previously. For the GST fusion protein of caveolin-1, the cDNA was subcloned into the pGEX-4T1 (*33*−*35*). The cDNAs encoding human c-Src in the pUSEamp CMV-based vector were purchased from Upstate Biotechnology, Inc. The cDNAs for wild-type PTP1B and the D181A mutant with a C-terminal HA tag were subcloned into pJ3H, as described previously (*15*). The PTP1B mouse monoclonal antibody and the HA monoclonal antibody (Y-11) were purchased from BD Pharmingen and Santa Cruz, respectively. Caveolin-1 mAb 2297 and phosphocaveolin-1 (Y14) mAb were gifts of Dr. Roberto Campos-Gonzalez (BD Pharmingen). The synthesis of compound **I** (Figure 1) was described previously (*36*).



I

FIGURE 1: Chemical structure of compound **I**.

Cell Culture and Transfection. COS-7 cells were grown in DME supplemented with glutamine, antibiotics (penicillin and streptomycin), and 10% fetal calf serum (*35*). NIH 3T3 cells were grown in DME supplemented with glutamine, antibiotics (penicillin and streptomycin), and 10% donor bovine calf serum (*37*). Cells were transfected using the Effectene transfection reagent (Qiagen, Chasworth, CA), as per the manufacturer's instructions.

Expression and Purification of Recombinant PTPs. PTP1B were expressed in *Escherichia coli* and purified as described previously (*38*). Recombinant HePTP and the catalytic domains of SHP2 were expressed and purified as $(His)_6$ fusion proteins (*39*, *40*). The catalytic domains of LAR were expressed and purified as recombinant glutathione *S*-transferase (GST) fusion proteins (*41*). The intracellular fragment of LAR containing both of the PTP domains was cleaved off the fusion protein using thrombin.

Tyrosine-Phosphorylated GST-Caveolin-1. The GST-Cav-1 (1−101) fusion protein (in the vector pGEX-4T-1) was purified as described (*33*−*35*). This fusion protein was expressed into two different *E. coli* strains [either BL21-(DE3) for nonphosphorylated caveolin-1 or TKB1 for tyrosine-phosphorylated caveolin-1] (*30*). The TKB1 strain is a derivative of BL21(DE3) that harbors a plasmid-encoded IPTG-inducible tyrosine kinase gene (the Elk receptor tyrosine kinase domain; Stratagene, Inc., La Jolla, CA).

Phosphocaveolin-1 Dephosphorylation by PTPs. Purified nonphopshorylated and tyrosine-phosphorylated GST-caveolin-1 (1−101) fusion proteins were immobilized on glutathione−agarose beads. Each 60 μL of GST-caveolin-1 fusion protein was incubated with various purified recombinant PTPs at 100 nM in 100 μL of DMG buffer (50 mM 3,3-dimethylglutarate, 1 mM EDTA, ionic strength of 0.15 M NaCl, pH 7.0) for 5 min. After centrifugation at 5000 rpm for 3 min, the supernatant was discarded; then 2× SDS loading buffer was added to stop the reaction. After being boiled for 5 min, the supernatant was resolved using SDS−PAGE, followed by Western blotting using anti-phosphocaveolin-1 IgG and pan caveolin-1 IgG. For inhibitor studies, the PTP1B inhibitor was added to the purified GST-caveolin-1 fusion proteins on ice before incubation with different purified PTPs.

In Vivo PTP1B Inhibitor Study. NIH 3T3 cells were seeded in six-well dishes with culture media, as described above. Various concentrations of the PTP1B inhibitor were added to each well and incubated 4 h at 37 °C. Then, cells were lysed in boiling sample buffer, resolved by SDS−PAGE, and subjected to Western blotting.

Immunoblotting with Anti-Phosphocaveolin-1 Antibody. Cells were lysed in a boiling sample. Samples were then collected and boiled for a total of 5 min. Samples were homogenized using a 26-gauge needle and a 1 mL syringe.

Cellular proteins were resolved by SDS−PAGE (12% acrylamide) and transferred to nitrocellulose membranes (0.2 μm). Blots were incubated for 2 h in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.2% Tween 20) containing 1% bovine serum albumin. After three washes with TBST, membranes were incubated for 2 h with the primary antibody (∼1000-fold diluted in TBST) and for 1 h with horseradish peroxidase-conjugated goat anti-rabbit/mouse IgG (∼5000-fold diluted). Proteins were detected using an ECL detection kit (Amersham).

Immunoprecipitation/Western Blot of Caveolin-1 and PTP1B. COS-7 cells were transiently cotransfected with caveolin-1, alone or in combination with c-Src or HA-tagged PTP1B (wild type or D181A). Thirty-six hours posttransfection, the cells were processed for immunoprecipitation using protein A−Sepharose CL-4B (Amersham Pharmacia Biotech). Briefly, cells were lysed in immunoprecipitation buffer containing 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 60 mM octyl glucoside with phosphatase inhibitors (50 mM NaF, 30 mM sodium pyrophosphate, 100 mM sodium orthovanadate), and protease inhibitors (Boehringer Mannheim). Lysates were precleared by addition of 50 μL of a 1:1 slurry of protein A−Sepharose in TNET buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1% Triton X-100) containing 1 mg/mL bovine serum albumin. After 30 min at 4 °C, samples were centrifuged for 5 s at 15000*g*. The resulting supernatants were transferred to fresh tubes, and 50 μL of protein A−Sepharose was added together with anti-HA antibody (Y-11; Santa-Cruz Biotech). Samples were then incubated for an additional 3 h at 4 °C. Immunoprcipitates were washed five times with immunoprecipitation buffer, and samples were separated by 12.5% SDS−PAGE and transferred to nitrocellulose. Blots were then probed with a well-characterized mAb directed against caveolin-1 (cl.2297; BD transduction; a mouse mAb).

RESULTS

PTP1B Dephosphorylates Tyrosine-Phosphorylated Caveolin-1. To determine whether caveolin-1 can serve as a substrate for PTP1B, we prepared Tyr14-phosphorylated caveolin-1 as a GST fusion protein in an *E. coli* TKB1 strain that harbors a plasmid-encoded IPTG-inducible tyrosine kinase gene (the Elk receptor tyrosine kinase domain). A fixed amount of purified recombinant Tyr14-phosphorylated GST-caveolin-1 fusion protein was incubated for 5 min with 100 nM PTP1B or 100 nM catalytic domain of other PTPs (including HePTP, LAR, and SHP2). As shown in Figure 2A, only PTP1B was capable of catalyzing caveolin-1 dephosphorylation as determined with a monoclonal antibody that specifically recognizes the Tyr14-phosphorylated caveolin-1 (*30*). Since the intrinsic phosphatase activities of the catalytic domains of PTP1B, HePTP, LAR, and SHP2 are similar when measured with the small molecule substrate *p*-nitrophenyl phosphate (*40*, *42*−*44*), the results indicate that PTP1B is highly specific for Tyr14 in caveolin-1. Further studies showed a time-dependent dephosphorylation of GST-caveolin-1 by 25 nM PTP1B, resulting in over 90% reduction in the Tyr14 phosphorylation level after 1 min of incubation (Figure 2B). Densitometry analysis of the time dependency of the PTP1B-catalyzed dephosphorylation of phosphocaveolin-1 yielded a k_{cat}/K_m of 4.2×10^6 M^{-1} s^{-1}, which is similar to those of the most efficient substrates for PTP1B (*45*). To exclude the possibility that the observed caveolin-1 dephosphorylation was caused by nonspecific phosphatases present in the recombinant PTP1B preparation, we tested if compound **I** (Figure 1), a potent and specific PTP1B inhibitor with a K_i value of 26 nM (*36*), could block dephosphorylation of phosphocaveolin-1 in the presence of PTP1B. As expected, compound **I** was capable of protecting phosphocaveolin-1 from dephosphorylation by PTP1B (Figure 3). Taken together, these results suggest that PTP1B is a highly efficient PTP for caveolin-1.

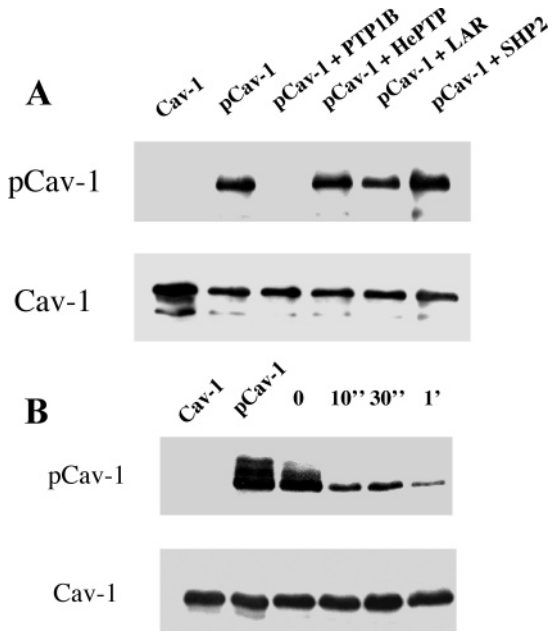


FIGURE 2: PTP1B is a specific and efficient phosphatase for phosphocaveolin-1. (A) Specificity of PTP1B for phosphocaveolin-1. Phosphocaveolin-1 was incubated with 100 nM purified recombinant PTP1B and the catalytic domains of HePTP, LAR, and SHP2 in assay buffer (pH 7.0) at room temperature for 5 min. Samples were then resolved by SDS−PAGE and blotted with phosphocaveolin-1 and caveolin-1 antibodies. (B) Dephosphorylation of caveolin-1 by PTP1B occurs in a time-dependent manner. Phosphocaveolin-1 was mixed with 25 nM purified PTP1B and incubated at 30 °C for different time periods. Equal volumes of aliquots were removed and subjected to SDS−PAGE/Western blot analysis with anti-phosphocaveolin-1 and anti-caveolin-1 antibodies.

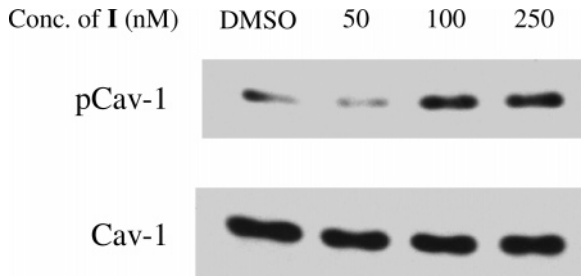


FIGURE 3: Compound **I** blocks the dephosphorylation of phosphocaveolin-1 by PTP1B in a concentration-dependent manner. Aliquots of phosphorylated caveolin-1 were incubated with different concentrations of compound **I** on ice. 25 nM purified PTP1B was added to the mixture and incubated at 30 °C for 1 min. Each sample was then subjected to SDS−PAGE/Western blot analysis with anti-phosphocaveolin-1 and anti-caveolin-1 antibodies.

*Inhibition of PTP1B with Compound **I** Increases Caveolin-1 Phosphorylation in the Cell.* Compound **I** and several of its derivatives represent the most potent and selective PTP1B inhibitors reported to date, which exhibit several

orders of magnitude of selectivity in favor of PTP1B against a panel of PTPs (*36*, *39*, *46*). Treatment of a number of insulin-sensitive cell lines with compound **I** markedly enhanced insulin receptor β (IRβ) and insulin receptor substrate 1 (IRS-1) phosphorylation, Akt and Erk1/2 activation, Glut4 translocation, and glucose uptake (*36*). Most importantly, the observed cellular effects (e.g., ~2-fold increase in IRβ and IRS-1 phosphorylation) with compound **I** are similar in magnitude to those observed with antisense-mediated reduction of PTP1B (*47*) and PTP1B knockout mice, which are resistant to diabetes and obesity (*5*). Collectively, these data demonstrate that compound **I** has the expected biological effects on insulin signaling and serves as both an insulin mimetic and a sensitizer, validating the notion that small molecule PTP1B inhibitors could be used as anti-diabetes therapeutics. Moreover, the data also establish the utility of potent and selective PTP1B inhibitors as reagents to study the roles of PTP1B in other signaling pathways.

To further analyze the role of PTP1B in controlling the level of caveolin-1 phosphorylation, we decided to examine the effect of PTP1B inhibition on the phosphorylation status of caveolin-1 in the cell. Using small molecule inhibitors, we established recently that PTP1B promotes integrin-mediated responses in fibroblasts by dephosphorylating the inhibitory pTyr527 and thereby activating the Src kinase (*12*). If caveolin-1 is a direct substrate of PTP1B, then we should observe an increase in caveolin-1 tyrosine phosphorylation when the cells are treated with compound **I**. On the other hand, if caveolin-1 is not a PTP1B substrate, two possible outcomes may be expected. In the first, there will be no change in caveolin-1 tyrosine phosphorylation status. In the second scenario, there will be a decrease in caveolin-1 tyrosine phosphorylation levels. The latter prediction is based on the following: (1) caveolin-1 is known to be a major substrate of the Src kinase (*25*−*27*, *30*), and (2) Src is activated by PTP1B (*12*). Consequently, it is possible that PTP1B inhibition by compound **I** may result in a decrease in the phosphorylation levels of Src substrates. Indeed, treatment of cells with compound **I** has been shown to lead to dose-dependent decreases in tyrosine phosphorylation of two Src substrates FAK and p130^{Cas}, indicating that neither FAK nor p130^{Cas} are direct substrates of PTP1B (*12*).

To determine if caveolin-1 is a PTP1B substrate, NIH 3T3 cells were exposed to compound **I** over a selected range of concentrations for 4 h. Cell lysates were then resolved by SDS−PAGE, electrotransferred to nitrocellulose membranes, and probed with anti-phosphocaveolin and pan-caveolin antibodies. As shown in Figure 4, NIH 3T3 cells treated with compound **I** clearly showed increased tyrosine phosphorylation of caveolin-1 in a concentration-dependent manner, indicating a role for PTP1B in the dephosphorylation of endogenous caveolin-1. Thus, the results with a potent and selective PTP1B inhibitor provide further evidence that the tyrosine-phosphorylated caveolin-1 is a cellular substrate of PTP1B.

PTP1B Associates with Caveolin-1 in the Cell. To provide further evidence that caveolin-1 is a substrate for PTP1B, we next performed substrate-trapping experiments using a PTP1B mutant (PTP1B/D181A) that is capable of binding protein substrate but unable to carry out the dephosphorylation reaction (*14*, *15*). PTP1B substrates identified using this approach include insulin receptor (*48*), EGF receptor (*14*, *15*), and JAK2 (*49*).

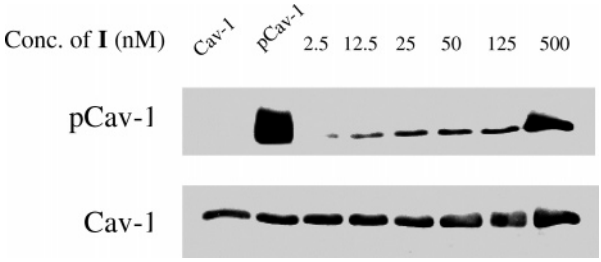


FIGURE 4: PTP1B inhibitor **I** causes the accumulation of phosphocaveolin-1 in NIH 3T3 cells. NIH 3T3 cells were seeded into six-well dishes. Different concentrations of compound **I** were added to each well; then the dishes were incubated in 37 °C for 4 h. Cell lysates were analyzed by SDS−PAGE/Western blot with anti-phosphocaveolin-1 and anti-caveolin-1 antibodies.

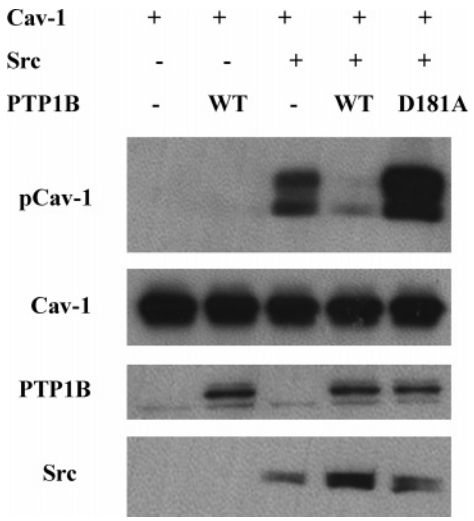


FIGURE 5: PTP1B expression decreases the Src-induced phosphorylation of caveolin-1 in the cell. COS-7 cells were transiently transfected with caveolin-1, c-Src, and PTP1B (wild type or substrate-trapping mutant). Thirty-six hours after transfection, the cells were collected, lysed, and subjected to SDS−PAGE/Western blot analysis.

We transiently cotransfected COS-7 cells with caveolin-1, alone or in combination with Src or HA-tagged wild-type PTP1B or the PTP1B substrate-trapping mutant D181A. The cell lysates were then fractionated by SDS−PAGE and immunoblotted with anti-caveolin-1 and anti-phosphocaveolin-1 antibodies (Figure 5). Consistent with previous findings, Src expression led to caveolin-1 phosphorylation on Tyr14. Efficient dephosphorylation of caveolin-1 occurred by expression of wild-type PTP1B. In contrast, the substrate-trapping mutant D181A was unable to dephosphorylate phosphocaveolin-1. In fact, the phosphorylation level of caveolin-1 was higher in cells expressing both Src and PTP1B/D181A than that in cells expressing only Src. These results suggest that the PTP1B substrate-trapping mutant binds to and protects caveolin-1 from dephosphorylation by endogenous PTPs.

To further investigate the association of PTP1B with caveolin-1, we performed immunoprecipitation experiments. Caveolin-1 and HA-tagged wild-type PTP1B or the PTP1B trapping mutant were cotransfected with or without c-Src expression plasmids in COS-7 cells. Then, the cells were lysed, and PTP1B was immunoprecipitated with anti-HA

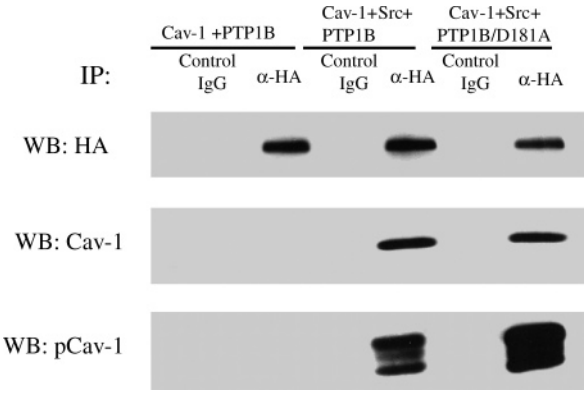


FIGURE 6: PTP1B co-immunoprecipitates with caveolin-1. Caveolin-1, c-Src, and HA-tagged PTP1B (wild type or substrate-trapping mutant) were transiently transfected into COS-7 cells. Thirty-six hours after transfection, cell lysates were incubated with anti-HA antobodies (to retrieve PTP1B), and the co-immunoprecipitated proteins were analyzed by SDS−PAGE and Western blotting.

antibodies. The immunoprecipitated proteins were then subjected to SDS−PAGE and immunoblotted to detect the presence of PTP1B, caveolin-1, and its tyrosine phosphorylation level. As shown in Figure 6, caveolin-1 was co-immunoprecipitated by both the wild-type PTP1B and the PTP1B substrate-trapping mutant. However, it is clear that the Tyr14 phosphorylation level in caveolin-1 associated with PTP1B/D181A is much higher than that in the complex between caveolin-1 and wild-type PTP1B, indicating again that the substrate-trapping mutant protects pTyr14 from dephosphorylation by endogenous PTP1B. Collectively, results from both substrate protection and substrate pull-down experiments support our conclusions from biochemical and PTP1B inhibitor studies that phosphocaveolin-1 is a novel PTP1B substrate.

DISCUSSION

PTP1B has been suggested to play a role in a number of signaling pathways, including insulin, EGF, and Src-mediated events (*5*, *6*, *10*−*16*). Although there is evidence that PTP1B can dephosphorylate insulin receptor, EGF receptor, and Src, it is not clear whether PTP1B can also directly control the phosphorylation levels of protein substrates downstream of these protein tyrosine kinases. A crucial step forward to enhance our understanding of PTP1B in modulating various cellular pathways is the identification of substrates downstream of the initial signaling events. Identification and characterization of specific PTP1B/substrate interactions will reveal new functions for PTP1B, as well as implicate PTP1B in novel signaling events. In this study, we have identified caveolin-1 as a new substrate for PTP1B.

Caveolin-1 is the principal component of caveolae and serves as a scaffolding protein for the efficient integration of multisignaling events (*21*, *22*, *24*). Caveolin-1 is a major substrate for Src, which phosphorylates caveolin-1 on tyrosine 14 (*25*−*27*). Tyr14 in caveolin-1 can also be phosphorylated in response to EGF or insulin stimulation (*28*, *30*, *31*). Although the functional significance of caveolin-1 tyrosine phosphorylation is still under investigation, it has been proposed that caveolin-1 phosphorylation on tyrosine 14 can serve as a docking site for SH2/PTB domain-containing proteins, thereby recruiting proteins into caveolae to activate downstream signaling cascades. In support of this hypothesis, the adaptor molecule Grb7 has been shown to interact with caveolin-1 in a tyrosine 14 phosphorylation dependent manner (*30*). Furthermore, binding of Grb7 to Tyr14-phosphorylated caveolin-1 augments anchorage-independent growth and EGF-stimulated cell migration (*30*). More recently, the C-terminal Src kinase (CSK) was also shown to bind caveolin-1 in a tyrosine 14 phosphorylation dependent manner (*50*). CSK negatively regulates the activities of Src family kinases by phosphorylating a conserved inhibitory tyrosine residue (527 in Src) in the C-terminus. Tyr14 phosphorylation of caveolin-1 promotes CSK translocation into caveolae, which may induce a feedback loop that leads to inactivation of the Src family kinases that are highly enriched in caveolae. Therefore, phosphorylation of caveolin on tyrosine 14 may be an intermediate step in the signaling cascade that occurs within caveolae. Finally, Tyr14-phosphorylated caveolin-1 has been shown to be required for integrin-regulated membrane domain internalization, which mediates inhibition of ERK, PI(3)K, and Rac (*51*). This finding implicates a novel mechanism for growth and tumor suppression by caveolin-1.

Since both PTP1B and caveolin-1 appear to participate in the same signal transduction pathways, we sought to investigate the possibility that PTP1B may directly regulate the phosphorylation status of caveolin-1. We show that PTP1B can efficiently catalyze the removal of the phosphoryl group from phosphocaveolin-1, while a number of PTPs (HePTP, LAR, and SHP2) are unable to dephosphorylate caveolin-1. Overexpression of PTP1B decreases the level of tyrosine 14 phosphorylation, while expression of the substrate-trapping mutant PTP1B/D181A causes the accumulation of phosphocaveolin-1 and prevents its dephosphorylation by endogenous PTPs. We further demonstrate that PTP1B physically associates with caveolin-1 in the cell. Finally, we show that inhibition of PTP1B activity with a potent and specific small molecule inhibitor blocks the PTP1B-catalyzed caveolin-1 dephosphorylation both in vitro and in vivo. These results strongly suggest that PTP1B can directly mediate the dephosphorylation of Tyr14 in caveolin-1 and that caveolin-1 is a specific substrate for PTP1B. The identification of caveolin-1 as a PTP1B substrate should provide an important stepping stone for future studies to gain a broader understanding of how PTP1B participates in various signaling processes.

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