The Role of the C-terminal Domain of Protein Tyrosine Phosphatase-1B in Phosphatase Activity and Substrate Binding*

Kristen M. Picha, Smita S. Patel, Sreekala Mandiyan, James Koehn, and Lawrence P. Wennogle

From Metabolic and Cardiovascular Diseases Research, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts 02139

Protein tyrosine phosphatase 1B (PTP-1B) has been implicated in the regulation of the insulin receptor. Dephosphorylation of the insulin receptor results in decreased insulin signaling and thus decreased glucose uptake. PTP-1B−/− mice have increased insulin sensitivity and are resistant to weight gain when fed a high fat diet, validating PTP-1B as a potential target for the treatment of type 2 diabetes. Many groups throughout the world have been searching for selective inhibitors for PTP-1B, and most of them target inhibitors to PTP-1B-(1–298), the N-terminal catalytic domain of the enzyme. However, the C-terminal domain is quite large and could influence the activity of the enzyme. Using two constructs of PTP-1B and a phosphopeptide as substrate, steady state assays showed that the presence of the C-terminal domain decreased both the $K_m$ and the $k_{cat}$ 2-fold. Pre-steady state kinetic experiments showed that the presence of the C-terminal domain improved the affinity of the enzyme for a phosphopeptide 2-fold, primarily because the off-rate was slower. This suggests that the C-terminal domain of PTP-1B may contact the phosphopeptide in some manner, allowing it to remain at the active site longer. This could be useful when screening libraries of compounds for inhibitors of PTP-1B. A compound that is able to make contacts with the C-terminal domain of PTP-1B would not only have a modest improvement in affinity but may also provide specificity over other phosphatases.

PROTEIN TYPHOSE PHOSPHATASES (PTPs)2 are essential components of signal transduction cascades. They work in conjunction with tyrosine kinases to control the phosphorylation of proteins, thereby regulating processes such as cell growth and metabolism (1–3). The family of PTPs is rather extensive, as there have been more than 100 human genes identified as protein tyrosine phosphatases via sequence analysis of the human genome (2). There is mounting interest in this family of enzymes, and different PTPs have been implicated in human diseases, including diabetes, autoimmunity, and cancer (4, 5).

PTP-1B has been linked to type 2 diabetes through negative regulation of the insulin-signaling pathway, as indicated by neutralizing antibodies (6), a knock-out mouse model (7, 8), antisense studies (9, 10), human clinical correlates (11, 12), and a variety of in vitro studies (13–19). This information has led to increasing interest in understanding PTP-1B at the molecular level and an intense effort to find inhibitors as potential drugs for type 2 diabetes (20).

PTP-1B is a member of the intracellular PTP family that comprises monomeric enzymes containing a single catalytic domain and various additional domains believed to be essential for cellular localization or enzymatic regulation (21–23). The full-length PTP-1B enzyme is made up of 435 amino acids, including an N-terminal 298-amino acid catalytic phosphatase domain and a C-terminal domain (amino acids 299–435) involved in protein-protein interactions and cellular localization (24, 25). For practical reasons, most studies on PTP-1B have employed a 298- or 321-amino acid enzyme fragment, devoid of the C terminus (26–31). However, no systematic study has verified that the enzymatic activity of this fragment is the same as the full-length enzyme. A recent study showed that an allosteric inhibitor of PTP-1B was 2-fold more potent on a more full-length construct relative to the catalytic domain alone (32).

The current study investigated whether the C-terminal domain of PTP-1B contributes to the catalytic function of the enzyme. PTP-1B-(1–411) was used to mimic the full-length enzyme, and PTP-1B-(1–298) contained only the catalytic domain. Steady state phosphatase assays and pre-steady state substrate binding assays revealed a difference between these two enzymes in their affinity for a phosphorylated peptide substrate. Specifically, the phosphorylated peptide was retained longer by PTP-1B-(1–411) compared with PTP-1B-(1–298), suggesting that the C-terminal domain of PTP-1B helps to stabilize the enzyme-substrate complex.

EXPERIMENTAL PROCEDURES

Enzymes—Human PTP-1B (amino acids 1–411 or 1–298) was cloned by PCR from a human hippocampal cDNA library (Clontech) and inserted into a pET 19-b vector (Novagen) at the NcoI restriction site. The sequence of the forward primer used to clone both PTP-1B-(1–411) and PTP-1B-(1–298) was GCC CGC CAT GGA GAT GGA AAA GGA G. The sequence of the

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Intra-Cellular Therapies, Inc. (ITI), Audubon Biomedical Science and Technology Park, 3960 Broadway, NY, NY 10032. Tel.: 212-923-3344 (ext. 210); Fax: 212-923-3388; E-mail: l.wенногл@intraacellulartherapies.com.

2 The abbreviations used are: PTP, protein tyrosine phosphatase; pNPP, para-nitrophenyl phosphate; pNP, para-nitrophenol; MES, 4-morpholineethanesulfonic acid.
were resuspended in lysis buffer (50 mM Tris-Cl, 100 mM NaCl, bacterial pellet was collected by centrifugation and the cells containing ampicillin (50 µg/ml). Expression of PTP-1B was initiated by induction with 1 mM isopropyl β-D-thiogalactopyranoside after the culture had reached an A600 of 0.6. After 4 h, the bacterial pellet was collected by centrifugation and the cells were resuspended in lysis buffer (50 mM Tris-Cl, 100 mM NaCl, 5 mM dithiothreitol, 0.1% Triton X-100, 100 µM phenylmethylsulfonyl fluoride, 100 µg/ml DNase I, 2 mM MgCl2, 100 µg/ml lysozyme, 1 tablet/50 ml complete protease inhibitors (Roche Diagnostics), pH 7.6). The cells were lysed by sonication, and the lysate was centrifuged at 100,000 × g for 60 min. The supernatant was collected, the buffer was exchanged with a cation exchange buffer (50 mM MES, 75 mM NaCl, 5 mM dithiothreitol, pH 6.5), loaded onto a POROS 20SP column (PerSeptive Biosystems, Inc.), and the protein was eluted using a linear NaCl gradient (0–500 mM). The PTP-1B protein was purified on a Source 30 Q column (Amersham Biosciences) using a linear NaCl gradient (0–300 mM). PTP-1B was pooled and frozen at −80 °C. As judged by SDS-polyacrylamide gel electrophoresis, the preparations were >95% pure. The protein concentration was determined by absorbance measurements at 280 nm in 8 M urea (extinction coefficient is 60,000 M⁻¹cm⁻¹ for PTP-1B-(1–411) and 45,900 M⁻¹cm⁻¹ for PTP-1B-(1–298)).

Phosphopeptide—The sequence of the phosphopeptide used in the experiments mimicked a sequence of insulin receptor substrate 1 (IRS-1) that is a substrate of PTP-1B (GNGD-pYMPMSPKS) (33). The peptide was synthesized using a RINK resin (Midwest Bio-Tech, Fishers, IN) using standard FMOC N-(9-fluorenylmethoxycarbonyl) solid phase peptide chemistry and purified using reversed-phase high pressure liquid chromatography. Amino acid analysis was performed (Commonwealth Biotechnologies, Inc.) for accurate determination of the peptide concentration.

pNPP Phosphatase Assay—The para-nitrophenyl phosphate (pNPP) phosphatase assays were carried out at room temperature in a cuvette (650 µl) using a LS50B PerkinElmer fluorometer. PTP-1B-(1–411) or PTP-1B-(1–298) (4 nM) was mixed with the phosphorylated peptide (0–150 µM) in Buffer A. After excitation at 280 nm, the fluorescence of the phosphorylated tyrosine was measured at 305 nm every 10 s for 300 s (34). The data were plotted and analyzed as described above. To ensure that less than 10% of the substrate was converted to product, several reactions were allowed to proceed to saturation. This allowed for conversion from fluorescence units to micromolar product. Experiments using the same peptide, but phosphorylated with 32P, showed that PTP-1B converted 100% of the substrate to product (data not shown).

Stopped Flow Peptide Binding—The pre-steady state peptide binding studies were done at 22 °C using a stopped flow instrument (KinTek Corp., State College, PA). PTP-1B-(1–411) or PTP-1B-(1–298) (1 µM final concentration) in Buffer A was rapidly mixed with phosphorylated peptide (0–40 µM final concentration) in Buffer A. Fluorescence was monitored using an excitation wavelength of 280 nm and measuring the fluorescence changes that occurred between 330–354 nm. For each experiment, at least five traces were averaged. No fluorescence changes were observed in the control experiments, including experiments done in the absence of PTP-1B or the phosphorylated peptide.

To accurately measure the observed rate at which peptide bound to PTP-1B, 500 data points were collected over six half-lives of the first phase of increasing fluorescence. After the first phase of changing fluorescence was saturated, there were additional phases of changing fluorescence. However, the rates for these additional phases were slower than the steady state rate of PTP-1B activity, indicating that they were not occurring during the first turnover. Therefore, only the first phase of changing fluorescence was monitored. At each concentration of peptide tested, the first phase of changing fluorescence was fit to an equation describing a single exponential to obtain the observed rate for peptide binding, k = F = a t⁻koff t + C, where F is the fluorescence as a function of time (t), a is the amplitude of the fluorescence change, and kobs is the observed rate constant of the fluorescence change. C is a constant.

The observed rate constant for peptide binding was then plotted as a function of the peptide concentration, and the data were fit to a straight line to obtain estimates for the intrinsic on and off rates for peptide binding to PTP-1B, kobs = k_on* (peptide) + k_off.

RESULTS

Two PTP-1B enzymes were characterized, PTP-1B-(1–411) and PTP-1B-(1–298), to investigate whether the C terminus of PTP-1B affects the enzymatic activity of the N-terminal domain. PTP-1B-(1–411) lacked the C-terminal 24 residues that localize the enzyme in the endoplasmic reticulum (Fig. 1A) and was used to mimic the full-length enzyme, because it was possible to obtain large quantities by overexpression in bacteria. PTP1B-(1–298) consisted of the catalytic domain alone. The enzymes were routinely purified to >95% purity, as indicated in Fig. 1.

Steady State Hydrolysis of pNPP—pNPP is commonly used as a substrate of tyrosine phosphatases. It changes its absorbance
when it is converted to product (pNP) and therefore can be monitored relatively easily. Steady state hydrolysis of pNPP by PTP-1B-(1–411) and PTP-1B-(1–298) was monitored over time by measuring the absorbance of pNP at 405 nm. As the concentration of pNPP was increased, the rate of the reaction increased in a hyperbolic manner. The rate of the reaction was plotted versus the concentration of substrate and was fit to a hyperbola to obtain Michaelis-Menten constants. The $k_{cat}$ and the $K_m$ for pNPP were similar for both forms of the enzyme, 2.8 s$^{-1}$ and 1.8 mM, respectively (Fig. 2A and Table 1).

**Steady State Hydrolysis of a Phosphopeptide**—The $K_m$ of a peptide containing a phosphotyrosine residue is smaller than the $K_m$ of pNPP or phosphotyrosine alone, indicating that there are additional residues besides those found directly in the active site of PTP-1B that contribute to recognition of the peptide substrate (28, 35). Additional steady state phosphatase assays were done using a phosphorylated peptide (GNGDpYMPMSPKS) as substrate, measuring the fluorescence change of the tyrosine residue upon dephosphorylation (excitation wavelength 280 nm, emission wavelength 305 nm) (34). In contrast to what was observed using pNPP as a substrate, PTP-1B-(1–411) and PTP-1B-(1–298) showed differences for both the $k_{cat}$ and the $K_m$ (Fig. 2B and Table 1). Although PTP-1B-(1–298) hydrolyzed the phosphorylated peptide with a $k_{cat}$ that was 2-fold faster when compared with PTP-1B-(1–411), the $K_m$ for PTP-1B-(1–298) was 2-fold lower.

**Pre-steady State Peptide Binding**—Pre-steady state stopped flow experiments were designed as shown in Fig. 3A. Briefly, PTP-1B was rapidly mixed in a stopped flow instrument with phosphorylated peptide, and a fluorescence change was measured.
ured in real time during peptide binding. It was not clear whether the fluorescence signal was a result of changes within PTP-1B or the phosphorylated peptide. However, the fluorescence signal increased upon peptide binding. The experiment was repeated at multiple peptide concentrations to elucidate the intrinsic rate constants (Fig. 3B). At each peptide concentration tested, the data were fit to an equation describing a single exponential function to obtain the observed rate for peptide binding. Fig. 3B shows data collected with PTP-1B-(1–411), but the same type of fluorescence changes were observed with PTP-1B-(1–298). The observed rate constants for peptide binding to PTP-1B increased linearly with peptide concentration, providing values for $k_{on}$ and $k_{off}$ ($2.0 \mu M^{-1} s^{-1}$ and $116.6 s^{-1}$, respectively).

![FIGURE 3. A](image)

**FIGURE 3.** A, the schematic shows the design of the stopped flow experiments. A motor drive rapidly pushes the contents of two syringes through a flow cell into a third syringe, which stops the flow and mixes both solutions in the flow cell. The reaction occurring in the flow cell can be observed by fluorescence measurements (excitation wavelength 280 nm, emission between 330–354 nm). B, PTP-1B-(1–411) (1 \mu M final concentration) was loaded into one syringe and peptide (0–40 \mu M) was loaded into the other syringe. Equal volumes from each syringe were rapidly mixed, and the resulting time courses of changes in PTP-1B-(1–411) fluorescence at increasing peptide concentrations are shown. The data were fit to an equation describing a single exponential (see “Experimental Procedures”). The experiment was repeated three times, and a representative data set is shown.

![TABLE 2](image)

**TABLE 2**

Pre-steady state kinetic parameters for PTP-1B-(1–411) versus PTP-1B-(1–298)

|                  | $k_{on}$ (\mu M$^{-1}$ s$^{-1}$) | $k_{off}$ (s$^{-1}$) | $K_D$ (\mu M) |
|------------------|-------------------------------|---------------------|--------------|
| PTP-1B-(1–411)   | 1.5 ± 0.1                     | 58.8 ± 4.6          | 39.2         |
| PTP-1B-(1–298)   | 1.4 ± 0.4                     | 119.6 ± 8.4        | 85.4         |
DISCUSSION

Despite the extensive mechanistic and structural data collected for tyrosine phosphatases, there are many questions that remain elusive. It was shown that PTP-1B resides at the cytoplasmic face of the endoplasmic reticulum, yet there have been additional reports localizing PTP-1B in microsomes or even cytoplasm. The insulin receptor is functional at the plasma membrane; therefore, it is unclear where PTP-1B acts upon the insulin receptor. In addition, there are many biological functions attributed to PTP-1B. It has been implicated in a variety of biological pathways, including insulin signaling, leptin signaling, platelet activation, cytokine signaling, integrin-mediated signaling, p210 bcr-abl-induced transformation, and cell cycle regulation. In light of these multiple functions of PTP-1B, it is not clear what the consequences may be of inhibiting such an enzyme. Finally, there have been reports of various forms of PTP-1B derived from alternative splicing and post-translational proteolytic cleavage, raising the question as to the biologically relevant form. It is quite likely that each of these questions has a different answer depending upon the cell type studied, resulting in a tremendous amount of information yet to be learned about the physiological function of PTP-1B and its mechanism of regulation.

Because of the strong evidence that PTP-1B counter-regulates the insulin signal, there has been a concerted effort to find inhibitors of PTP-1B as therapeutic agents for type 2 diabetes. Curiously, many groups have relied upon recombinant PTP-1B forms lacking the C-terminal domain. The C terminus of PTP-1B has several striking features. First, it has a proline-rich region (amino acid residues 301–315) shown to interact with Src homology (SH3)-containing proteins such as p130Cas. It also has a hydrophobic tail (amino acid residues 400–435) that localizes PTP-1B to the endoplasmic reticulum. In addition, two serine residues (amino acid residues 352 and 386) become phosphorylated during mitosis and in response to stress. Taken together, these data suggest that the C terminus of PTP-1B is critical for enzyme regulation as well as cellular localization. An extensive search of proteins interacting with PTP-1B indicates that there are more than 50 documented interactions, including erythropoietin receptor, focal adhesion kinase, caveolin-1, and Janus kinase 2. In light of this information, and contrary to the view from numerous crystal structures of the enzyme catalytic core, it is impossible to envision PTP-1B inside a cell without including a host of binding interactions and protein complexes, some of which occur through the C terminus of the enzyme. But does the C terminus contribute to the phosphatase activity of the N-terminal domain? Can the N-terminal phosphatase domain be studied independently, or does the C terminus contribute to its catalytic activity? The answer to this question is particularly important for interpreting studies using recombinant PTP-1B in relation to in vivo predictions for potential drug candidates.

In this study, the steady state activity of two PTP-1B enzymes was compared. One of the enzymes consisted of only the catalytic domain (1–298) and the other enzyme was used to mimic the full-length protein (1–411). Interestingly, the steady state experiments using a phosphopeptide showed that the $K_m$ was 2-fold higher in the absence of the C terminus, yet the $k_{cat}/K_m$ was 2-fold faster (Table 1). The $K_m$ is often thought to be equivalent to $K_D$, but it is a macroscopic constant and in this case it is unlikely to be equivalent to the $K_D$. Because there are so many reaction intermediates that occur as PTP-1B dephosphorylates a peptide, the $K_m$ is likely lower than the $K_D$ in addition. In the reaction intermediates at the catalytic domain of PTP-1B, the apparent on-rate is not likely to be equivalent to the intrinsic on-rate. The only time the $k_{cat}/K_m$, an apparent second-order rate constant, can be considered equal to the true microscopic on-rate is when substrate binding is the rate-limiting step of the catalytic reaction. This is likely not the case for PTP-1B. There are many conformational changes that must occur after binding, including closure of the WPD loop (amino acids 179–181), formation and hydrolysis of a covalent phosphopeptide intermediate, release of the dephosphorylated peptide, and release of the phosphate. The $k_{cat}/K_m$ in this case is likely the lower limit for the true on-rate.

Pre-steady state stopped flow studies were initiated to directly measure the intrinsic rate constants for peptide binding to the two forms of PTP-1B. Interestingly, the measured on-rate was not so different from the apparent second-order rate constant (1.5 versus 0.8 $\mu$M$^{-1}$s$^{-1}$ for PTP-1B-(1–411) and 1.4 versus 1.0 $\mu$M$^{-1}$s$^{-1}$ for PTP-1B-(1–298)). However, the affinity of a phosphopeptide for the construct containing the C-terminal domain was 2-fold tighter (Table 2). The phosphorylated peptide substrate was retained on PTP-1B-(1–411) twice as long as it was on PTP-1B-(1–298), indicating that the C terminus lends conformational stability to the enzyme-peptide complex. Either there are amino acids in the C terminus that directly contribute to peptide binding or the presence of the C terminus influences the structure of the phosphatase domain, allowing the phosphatase domain to retain the peptide longer.

Considering the results and conclusions from the pre-steady state data, it is interesting to re-examine the steady state data. The $k_{cat}/K_m$ for the phosphorylated peptide was ~600-fold faster when compared with pNPP, regardless of which form of PTP-1B was used. This is primarily because of the significant difference in the $K_m$ values for the two substrates, confirming that the interactions between PTP-1B and the amino acids surrounding the tyrosine in the phosphorylated peptide contribute significantly to substrate recognition. Interestingly, the $k_{cat}$ was the same for both forms of the enzyme when pNPP was used as a substrate, but not when the phosphorylated peptide was used as a substrate (Fig. 2, Table 1). The small molecule pNPP binds directly in the active site of PTP-1B without making extensive interactions with the surface of the enzyme, whereas the phosphorylated peptide lays on the surface of PTP-1B, extending the phosphorylated tyrosine residue into the active site (26, 28, 30). This suggests that the C terminus does not profoundly influence the amino acids making contact with the substrate in the active site. Rather, the C terminus has a more global change in the conformation of the catalytic domain of PTP-1B, which in turn contributes to substrate recognition. The current understanding of the structure and function of PTP-1B is derived from a number of different sources and tech-
Role of C-terminal Domain of PTP-1B

niques, including a wealth of crystal structures of the catalytic domain, protein-protein interaction data, post-translational modifications, biochemical methods, and computer-aided predictions (30, 31, 63–65). The composite information from the abundant crystal structures defines the catalytic core of the enzyme (1–298) and the interaction of various substrates and inhibitors. These methods have defined binding sites and interactions in great detail, including the role of the PTP loop (216–221), the WPD loop (181–182), residues involved in substrate recognition (Lys-36, Val-49, Lys-120), and a secondary substrate site including Tyr-20, Arg-24, His-25, Phe-52, and Arg-254. The details of substrate interactions were elucidated by an iterative affinity selection method using non-hydrolyzing phosphotyrosine mimics of a substrate peptide derived from the epidermal growth factor receptor (61). Using this method, Pellegreti et al. found an interesting preference within peptide substrates for an aromatic residue immediately prior to the tyrosine, a site known to be in proximity to Arg-47 and Asp-48.

Recently, using a yeast-based selection system, Montalibet et al. (32) identified mutant enzymes that showed a significant resistance to difluoro-naphthalenyl methylphosphonic acid inhibitors. Mutant PTP-1B enzymes allowing for selection with this system included numerous point mutations at residues Glu-297, Ser-295, Trp-291, Ala-189, Ser-187, and Glu-186, a large preference for the far C-terminal region of the catalytic domain. The Ser-295 mutation was extensively characterized, and a theory of how this effect is carried to the active site that is 21 Å away was presented. The data indicate that there are extensive hydrogen bonds between two helices (helices 7 and 3), and disruption of these interactions likely results in structural changes to Phe-182, leading directly to the WPD loop.

Soc homology 3 domain proteins that interact at the C-terminal proline-rich area would bind to PTP-1B close to helix 7. As such, these interactions may influence enzyme activity by the hydrogen bond network through helix 3, as depicted by Montalibet et al. (32). One can easily envision a protein-protein interaction occurring immediately after the catalytic domain playing an important role in catalysis at the active site.

The presented data suggest that the C-terminal domain of PTP-1B is involved in more than subcellular localization and protein-protein interactions. It also participates in substrate recognition of the phosphatase domain. It is possible that there are direct contacts between the C-terminal domain of PTP-1B and phosphorylated protein substrates. Alternatively, the C-terminal domain may cause a global conformational change influencing the N-terminal domain, such that there are additional interactions between the N-terminal domain and phosphorylated substrate. Either way, these data could influence the way in which inhibitors of PTP-1B are screened. The catalytic domains of protein tyrosine phosphatases are rather conserved, and finding a selective inhibitor has been difficult. Using an enzyme that contains both the N- and C-terminal domains of PTP-1B may allow for modest improvements in affinity but a significant improvement in specificity.

Acknowledgments—We thank Iou-Iou Sytwu and Isodoros Vlattas for preparation of the phosphopeptide.

REFERENCES

1. Burke, T. R., and Zhang, Z. Y. (1998) Biopolymers 47, 225–241
2. Zhang, Z. Y. (2001) Curr. Opin. Chem. Biol. 5, 416–423
3. Wang, W. Q., Sun J. P., and Zhang, Z. Y. (2003) Curr. Top. Med. Chem. 3, 739–748
4. Ukkola O., and Santanieni, M. (2002) J. Intern. Med. 251, 467–475
5. Kennedy, B. P., and Ramachandran, C. (2000) Biochem. Pharmacol. 60, 877–883
6. Ahmad, F., Li, P. M., Meyerovitch, J., and Goldstein, B. J. (1995) J. Biol. Chem. 270, 20503–20508
7. Klaman, L. D., Boss, O., Peroni, O. D., Kim, J. K., Martino, J. L., Zabolotny, J. M., Moghal, N., Lubkin, M., Kim, Y., Sharpe, A. H., Stricker-Krongrad, A., Shulman, G. I., Neel, B. G., and Kahn, B. B. (2000) Mol. Cell. Biol. 20, 5479–5489
8. Elchebly, M., Payette, P., Michalszyn, E., Cromlish, W., Collins, S., Loy, A. L., Normandin, D., Cheng, A., Himms-Hagen, J., Chan, C. C., Ramachandran, C., Gresser, M. J., Tremblay, M. L., and Kennedy, B. P. (1999) Science 283, 1544–1548
9. Rondinone, C. M., Trevillian, J. M., Clampit, J., Gum, R. M., Berg, C., Kroeger, P., Frost, L., Zinker, B. A., Reilly, U. R., Ulrich, B., Monia, B. P., Irousek, M. R., and Waring, I. F. (2002) Diabetes 51, 2405–2411
10. Zinker, B. A., Rondinone, C. M., Trevillian, J. M., Gum, R. M., Clampit, J. E., Waring, J. F., Xie, N., Wilcox, D., Jacobson, P., Frost, L., Kroeger, P. E., Reilly, R. M., Koterski, S., Oppengen, T. J., Ulrich, R. G., Crosby, S., Butler, M., Murray, S. F., McKay, R. A., Bhanot, S., Monia, B. P., and Irousek, M. R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 11357–11362
11. Ahmad, F., Azevedo, J. L., Cortright, R., Dohn, G. L., and Goldstein, B. J. (1997) J. Clin. Investig. 100, 449–458
12. Wu, X., Hofstedt, J., Deeb, W., Singh, R., Sedkova, N., Zilbering, A., Zhu, L., Park, P. K., Arner, P., and Goldstein, B. J. (2001) J. Clin. Endocrinol. Metab. 86, 5973–5980
13. Ramachandran, C., Aebersold, R., Tonks, N. K., and Pot, D. A. (1992) Biochemistry 31, 4232–4238
14. Ahmad, F., and Goldstein, B. J. (1995) Biochim. Biophys. Acta 1248, 57–69
15. Ahmad, F., and Goldstein, B. J. (1995) Metabolism 44, 1175–1184
16. Ahmad, F., and Goldstein, B. J. (1995) Am. J. Physiol. 268, E932–E940
17. Kenner, K. A., Anyanwu, E., Olefsky, I. M., and Kusari, J. (1996) J. Biol. Chem. 271, 19810–19816
18. Seely, B. L., Staubs, P. A., Reichart, D. R., Berhanu, P., Milarski, K. L., Saltiel, A. R., Kusari, J., and Olefsky, J. M. (1996) Diabetes 45, 1379–1385
19. Byron, J. C., Kusari, A. B., and Kusari, J. (1998) Mol. Cell. Biochem. 182, 101–108
20. Taylor, S. D., and Hill, B. (2004) Expert Opin. Investig. Drugs 13, 199–214
21. Li, L., and Dixon, I.E. (2000) Semin. Immunol. 12, 75–84
22. Tonks, N. K., and Neel, B. G. (2001) Curr. Opin. Chem. Biol. 13, 182–195
23. Zhang, Z. Y. (1998) Crit. Rev. Biochem. Mol. Biol. 33, 1–52
24. Charbonneau, H., Tonks, N. K., Kumar, S., Diltz, C. D., Harrylock, M., Cool, D. E., Krebs, E. G., Fischer, E. H., and Walsh, K. A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5252–5256
25. Frangioni, J. V., Beahm, P. H., Shifrin, V., Jost, C. A., and Neel, B. G. (1992) Cell 68, 545–560
26. Groves, M. R., Yao, Z. J., Roller, P. P., Burke, T. R., Jr., and Barford, D. (1998) Biochemistry 37, 17773–17783
27. Iversen, L. F., Andersen, H. S., Moller, K. B., Olsen, O. H., Peters, G. H., Branner, S., Mortensen, S. B., Hansen, T. K., Lau, J., Ge, Y., Holsworth, D. D., Newman, M. J., and Moller, N. P. (2001) Biochemistry 40, 14812–14820
28. Salmeen, A., Andersen, J. N., Myers, M. P., Tonks, N. K., and Barford, D. (2000) Mol. Cell 6, 1401–1412
29. Bleasdale, J., Ev, D., Alpak, B. Z., Jacob, C. S., Swanson, M. L., Wang, X. Y., Thompson, D. P., Conradi, R. A., Mathews, W. R., Laborde, A. L., Stuchly, C. W., Heijbel, A., Bergdahl, K., Bannow, C. A., Smith, C. W., Svensson, C., Liljebjörn, C., Schostae, H. J., May, P. D., Stevens, F. C., and Larsen, S. D. (2001) Biochemistry 40, 5642–5654
30. Puiu, Y. A., Zhao, Y., Sullivan, M., Lawrence, D. S., Almo, S. C., and Zhang, Z. Y. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13420–13425
31. Iversen, L. F., Andersen, H. S., Branner, S., Mortensen, S. B., Peters, G. H.,...
Role of C-terminal Domain of PTP-1B

FEBRUARY 2, 2007 • VOLUME 282 • NUMBER 5

JOURNAL OF BIOLOGICAL CHEMISTRY

Norris, K., Olsen, O. H., Jeppesen, C. B., Lundt, B. F., Ripka, W., Moller, K. B., and Moller, N. P. (2000) J. Biol. Chem. 275, 10300–10307
32. Montalibet, J., Skorey, K., McKay, D., Scapin, G., Asante-Appiah, E., and Kennedy, B. (2006) J. Biol. Chem. 281, 5258–5266
33. Goldstein, B. J., Bittner-Kowalczyk, A., White, M. F., and Harbeck, M. (2000) J. Biol. Chem. 275, 4283–4289
34. Zhang, Z. Y., Maclean, D., Thieme-Sefler, A. M., Roeske, R. W., and Dixon, J. E. (1993) Anal. Biochem. 211, 7–15
35. Dadke, S., Kusari, J., and Chernoff, J. (2000) J. Biol. Chem. 275, 23642–23647
36. Frangioni, J. V., Oda, A., Smith, M., Salzman, E. W., and Neel, B. G. (1993) EMBO J. 12, 4843–4856
37. Haj, F. G., Verveer, P. J., Squire, A., Neel, B. G., and Bastiaens, P. I. (2002) Science 295, 1708–1711
38. Shi, K., Egawa, K., Maegawa, H., Nakamura, T., Ugi, S., Nishio, Y., and Kashiwagi, A. (2004) J. Biochem. 136, 89–96
39. Romsicki, Y., Reece, M., Gauthier, J., Asante-Appiah, E., and Kennedy, B. P. (2004) J. Biol. Chem. 279, 12868–12875
40. Burke, T. R., Kole, H. K., and Roller, P. P. (1994) Biochem. Biophys. Res. Commun. 204, 129–134
41. Egawa, K., Maegawa, H., Shimizu, S., Morino, K., Nishio, Y., Bryer-Ash, M., Cheung, A. T., Koliss, J. K., Kikkawa, R., and Kashiwagi, A. (2001) J. Biol. Chem. 276, 10207–10211
42. Zabolotny, J. M., Bence-Hanulec, K. K., Stricker-Krongrad, A., Haj, F., Wang, Y., Minokoshi, Y., Kim, Y. B., Wilmquist, J. K., Tartaglia, L. A., Kahn, B. B., and Neel, B. G. (2002) Dev. Cell 2, 489–495
43. Cheng, A., Uetani, M., Simonic, P. D., Chaubey, V. P., Lee-Loy, A., McGlade, C. J., Kennedy, B. P., and Tremblay, M. L. (2002) Dev. Cell 2, 385–387
44. Lund, I. K., Hansen, J. A., Andersen, H. S., Moller, N. P., and Billestrup, N. (2005) J. Mol. Endocrinol. 34, 339–351
45. Lerea, K. M., Tonks, N. K., Krebs, E. G., Fischer, E. H., and Glomset, J. A. (1989) Biochemistry 28, 9286–9292
46. Ezumi, Y., Takayama, H., and Okuma, M. (1995) J. Biol. Chem. 270, 11927–11934
47. Myers, M. P., Andersen, J. N., Cheng, A., Tremblay, M. L., Horvath, C. M., Parisien, J. P., Salmeen, A., Barford, D., and Tonks, N. K. (2001) J. Biol. Chem. 276, 47771–47774
48. Arregui, C. O., Balsamo, J., and Lilien, J. (1998) J. Cell Biol. 143, 861–873
49. Liu, F., Sells, M. A., and Chernoff, J. (1998) Curr. Biol. 8, 173–176
50. Cheng, A., Bal, G. S., Kennedy, B. P., and Tremblay, M. L. (2001) J. Biol. Chem. 276, 25848–25855
51. Liang, F., Lee, S. Y., Liang, J., and Chernoff, J. (2005) J. Biol. Chem. 280, 24857–24863
52. LaMontagne, K. R., Hannon, G., and Tonks, N. K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14094–14099
53. Flint, A. J., Gebbink, M. F., Franza, B. R., Hill, D. E., and Tonks, N. K. (1993) EMBO J. 12, 1937–1946
54. Schievella, A. R., Paige, L. A., Johnson, K. A., Hill, D. E., and Erikson, R. L. (1993) Cell Growth Differ. 4, 239–246
55. Shifrin, V. I., and Neel, B. G. (1993) J. Biol. Chem. 268, 25376–25384
56. Shifrin, V. I., Davis, R. J., and Neel, B. G. (1997) J. Biol. Chem. 272, 2957–2962
57. Cohen, J., Oren-Young, L., Klingmuller, U., and Neumann, D. (2004) Biochem. J. 377, 517–524
58. Zhang, Z., Lin, S., Neel, B., and Haimovich, B. (2006) J. Biol. Chem. 281, 1746–1754
59. Caselli, A., Mazzinghi, B., Camici, G., Manao, G., and Ramponi, G. (2002) Biochem. Biophys. Res. Commun. 296, 692–697
60. Pellegrini, M., Liang, H., Mandiyan, S., Wang, K., Yuryev, A., Vlattas, I., Sytwu, T., Li, Y., and Wennogle, L. (1998) Biochemistry 37, 15598–15606
61. Fersht, A. (1985) Enzyme Structure and Mechanism, 2nd Ed., W. H. Freeman and Company, New York
62. Barford, D., Flint, A. J., and Tonks, N. K. (1994) Science 263, 1397–1404
63. Jia, Z., Barford, D., Flint, A. J., and Tonks, N. K. (1995) Science 268, 1754–1758
64. Wiesmann, C., Barr, K., Kung, J., Zhu, J., Erlanson, D. A., Shen, W., Fahr, B. J., Zhong, M., Taylor, L., Randal, M., McDowell, R. S., and Hansen, S. K. (2004) Nat. Struct. Mol. Biol. 11, 730–737