Structure Determination of T Cell Protein-tyrosine Phosphatase*

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Protein-tyrosine phosphatase 1B (PTP1B) has recently received much attention as a potential drug target in type 2 diabetes. This has in particular been spurred by the finding that PTP1B knockout mice show increased insulin sensitivity and resistance to diet-induced obesity. Surprisingly, the highly homologous T cell protein-tyrosine phosphatase (TC-PTP) has received much less attention, and no x-ray structure has been provided. We have previously co-crystallized PTP1B with a number of low molecular weight inhibitors that inhibit TC-PTP with similar efficiency. Unexpectedly, we were not able to co-crystallize TC-PTP with the same set of inhibitors. This seems to be due to a multimerization process where residues 130–132, the DDQ loop, from one molecule is inserted into the active site of the neighboring molecule, resulting in a continuous string of interacting TC-PTP molecules. Importantly, despite the high degree of functional and structural similarity between TC-PTP and PTP1B, we have been able to identify areas close to the active site that might be addressed to develop selective inhibitors of each enzyme.

Protein-tyrosine phosphatases (PTPs)1 are key regulators of signal transduction processes (1, 2). The family of classical PTPs can be divided into two broad categories as intracellular and receptor-like PTPs covering a total of 17 subtypes (3). Receptor-like PTPs contain an extracellular domain, a single transmembrane domain, and one or two cytoplasmic PTP domains. Intracellular PTPs generally contain one PTP domain and an N- or C-terminal domain that targets the enzymes to specific subcellular localizations, as exemplified by the targeting of PTP1B to the endoplasmic reticulum (4).

PTP1B and TC-PTP are two closely related intracellular enzymes. PTP1B was the first protein-tyrosine phosphatase to be identified and characterized (5, 6). Shortly after this landmark event, PTP1B was cloned from a placenta cDNA library (7), and TC-PTP was cloned from a peripheral human T cell cDNA library (8). Despite its name, TC-PTP is ubiquitously expressed (9). Alternative splicing gives rise to two forms of TC-PTP that differ in the C termini, a 45-kDa form that is targeted to the nucleus and a 48-kDa form that localizes to the endoplasmic reticulum via a hydrophobic C-terminal region (10). TC-PTP is tightly regulated during the cell cycle and seems to play an important role in mitogenesis (9). In a recent study, it was shown that cellular stress causes reversible cytoplasmic accumulation of the 45-kDa form of TC-PTP (i.e. the nuclear form) (11).

Although they have a sequence identity of about 74% in the catalytic domains (see Fig. 1), TC-PTP and PTP1B clearly fulfill different biological functions, as has been demonstrated in knock-out mice. Thus, although PTP1B knock-out mice show increased insulin sensitivity and resistance to diet-induced obesity and are viable with a normal life span (12, 13), TC-PTP knock-out mice die at 3–5 weeks of age (14).

In accordance with these in vivo observations, substrate trapping experiments have further shown that PTP1B and TC-PTP recognize different cellular targets (15, 16). At present it is not known to which degree this is due to different inherent substrate specificity that resides within the catalytic domains or to other regulatory mechanisms. For example, the activity and function of PTP1B can be regulated at different levels, including transcription (17), alternative splicing (18), proteolytic processing (19), and covalent modification (i.e. phosphorylation of specific residues such as Ser-50) (20). Likewise, as indicated above, alternative splicing may determine which substrates are recognized by TC-PTP (i.e. nuclear substrates by the 45-kDa form and cytoplasmic substrates by the 48-kDa form). However, by comparing substrates trapped with PTP1B and those trapped by targeting a TC-PTP/PTP1B chimera to the endoplasmic reticulum, Tonks and coworkers (16) provide convincing evidence that at least part of the observed differences in substrate recognition capacity between the two enzymes is due to differences in intrinsic substrate specificity. Thus, fine structural differences not readily identifiable by primary sequence analyses may account for the observed differences in substrate recognition by PTP1B and TC-PTP. Similarly, elegant catalytic domain-swapping experiments of two other homologous PTPs, SHP-1 and SHP-2, clearly indicate that substantial substrate specificity may reside in the PTP domains (21, 22). In addition, areas outside the highly conserved regions surrounding the active sites may contribute to substrate binding (3).

Although the exact molecular mechanism(s) underlying the above phenotype of PTP1B knock-out mice still remains to be identified, these studies indicate that PTP1B could be an attractive drug target for treatment of type 2 diabetes. As a
The PTP1B and TC-PTP inhibitors have shown promise in disease treatments, with PTP1B inhibition being particularly effective in P41212 or P4 3212 with cell dimensions of 68.3, 68.3, and 129.7 Å, respectively. The two proteins exhibit a high degree of similarity, with the catalytic domains having 74% sequence identity. The study aimed to compare PTP1B and TC-PTP in order to identify selective inhibitors.

**EXPERIMENTAL PROCEDURES**

**Cloning, Expression, and Purification—**PTP1B 1–321 and TC-PTP 1–314 were cloned, expressed, and purified as described previously (24–26). The cDNAs encoding these PTPs were obtained by polymerase chain reaction using primers with convenient cloning sites and were inserted into the pET11a expression vector, and PTP1B and TC-PTP were expressed essentially as described previously (26). PTP1B and TC-PTP were purified in a two-step procedure. In brief, compound 4, which is a selective PTP1B/TC-PTP inhibitor (see Fig. 2), was coupled to epoxy-activated Sepharose 6B (Amersham Biosciences) according to the manufacturer’s instructions (100 mg of compound 4/g of drained column material). Lysates from Escherichia coli producing PTP1B and TC-PTP were cleared by centrifugation and applied to the column. The enzymes were eluted by a combined pH (6.2–9.0) and salt gradient (0.1–1.0 M NaCl), resulting in ~90% pure preparations. The final polishing consisted of an anion exchange purification step (Mono-Q, Amersham Biosciences). Before crystallization, buffer exchange was performed using a Superdex 200 column (Amersham Biosciences). Further experimental details will be published elsewhere.

**Determination of Kinetic Constants and Inhibitor Constants, Ki—**The phosphatase activity was determined using p-nitrophenyl phosphate as substrate essentially as described (24, 27) using a constant ionic strength three-component buffer at pH 6.5, which essentially as described (24, 27) using a constant ionic strength three-component buffer at pH 6.5 (28). Substrates were used at concentrations of 2 mM NaCl, 0.2 mM EDTA, and 3 mM dithiothreitol was used for crystal growth. Ni2+ ions were used for crystal growth. Ni2+ ions were used for crystal growth.

**Crystallization—**An ~10 mg/ml TC-PTP in 10 mM Tris, pH 7.5, 25 mM NaCl, 0.2 mM EDTA, and 3 mM dithiothreitol was used for crystallization. Crystals were grown by the hanging drop vapor diffusion method. Two μl of the PTP solution with 2 μl of reservoir solution consisting of 0.05–0.25 M Hapes buffer, pH 8.0, 0.2 M magnesium acetate, 20% polyethylene glycol 8000, and 0.1% β-mercaptoethanol. The reservoir volume was 1 ml. Crystals grew to the size of 0.5 × 0.3 × 0.1 mm over approximately 1 week, and three or more weeks in total were used for crystal growth.

**Data Collection—**Data were collected using a Mar345 image plate detector on a rotating anode (RU300, CuKα, 50 kV/100 μA) equipped with Osmic multilayer mirror system. The data collection was performed on a single crystal at room temperature. A data set to 2.53Å resolution was obtained. Data processing was performed using Denzo, Scalepack, and the CCP4 program suite (31, 32). From autoindexing and the systematic and absent reflections, the space group was determined to be P4 321, 2 or P4 321 with cell dimensions a = b = 60.5 Å and c = 187.6 Å. The Vm was calculated to be 2.3 Å3/dalton with a TC-PTP monomer in asymmetric unit (Vm = 2.3 Å3/dalton for the average protein crystal).

**Molecular Replacement Solution—**A molecular replacement solution was found using Amore (33) and PTP1B (Protein Data Bank code 1C88) as a search model (ligand and water molecules were omitted from the structure). The search was performed in both P4 321 and P4 321, 2 with the correct solution identified in P4 321, 2.

**Refinements—**All refinements were performed using CNS version 2000 (Accelrys). Interchanging cycles of model building using X-build (Accelrys) and refinement were performed. To avoid phase bias from the molecular replacement search model, 5% of the amplitudes were omitted from the refinements and used for R-Free calculations, and the lowering of R-free was monitored during all refinements.

**RESULTS**

The 1–321 PTP1B construct is the best characterized enzyme domain within the PTP family, and its activity and structure have been studied in numerous reports since its original identification and cloning. Therefore, to compare directly with this gold standard in the PTP field we decided to clone and express the similar construct of TC-PTP, i.e. residues 1–314.

The sequence identity between the catalytic domains of TC-PTP and PTP1B is 74% (Fig. 1) with the major differences clustered in four stretches of amino acid residues, (i) 1-29, (ii) 129–148, (iii) 158–174, and (iv) 235–246 (TC-PTP numbering is used).

**Enzyme Kinetics—**To compare PTP1B and TC-PTP at the functional level, we first determined the steady state kinetic parameters, kcat and Km for each enzyme using p-nitrophenyl phosphate as substrate. As shown in Table I, the catalytic efficiencies of these two enzymes are almost identical, thus providing an initial indication that the high degree of primary sequence identity also translates into a high degree of similarity at the structural and functional levels.

**Probing the Active Site Cavity with Inhibitors—**We next probed the active site cavities of the two enzymes with a set of PTP inhibitors. We have previously shown that 2-isoninylbenzoic acid (Fig. 2, compound 1) is a general PTP inhibitor with the two carboxyl groups interacting with conserved residues in the active sites of each enzyme (26). Of note, in the present context, we also found that an additional ring system could dramatically change the inhibitory profiles of the inhibitors.

| Statistics of x-ray data and refinements |
|-----------------------------------------|
| Space group                             |
| P4 321                                  |
| Unit cell parameters                    |
| a = b = 60.5 Å                          |
| c = 187.6 Å                            |
| Completeness (20–2.56 Å)                |
| 97.5%                                   |
| Completeness (2.6–2.56 Å)              |
| 82.0%                                   |
| Multiplicity (20–2.56 Å)                |
| 3.3                                     |
| Rmerge (20–2.56 Å)                      |
| 6.7%                                    |
| Rmerge (2.6–2.56 Å)                     |
| 38.1%                                   |

| Table I |
|---------|
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these compounds. Thus, although the naphthyl-based compound 2 is a relatively potent inhibitor of PTP-LAR at pH 5.5, the indole-based compound 3 is a poor inhibitor of this enzyme. Conversely, compound 3 is a relatively potent inhibitor of SHP-1, whereas compound 2 is about 3-fold less potent. Accordingly, this shows that even very close to the active site, structural differences among PTPs can be detected with these low molecular weight inhibitors.

As shown in Table III, only minor differences could be demonstrated when compounds 1–4 were analyzed for inhibitory activity against PTP1B and TC-PTP. This is in agreement with our recent findings where two pyran-based inhibitors were found to inhibit these two enzymes with similar efficiency (24). Thus, we conclude that, from a functional point of view, the catalytic clefts of PTP1B and TC-PTP are very similar.

**Crystallization of TC-PTP**—Although the above enzyme kinetic characterization and inhibitor studies as well as molecular modeling (not shown) clearly indicate that the catalytic domains of PTP1B and TC-PTP must be very similar, protein x-ray crystallography is needed for unequivocal structural comparison of the two enzymes. We first attempted to co-crystallize TC-PTP with several of the above ligands that were found to inhibit PTP1B and TC-PTP with similar potency and for which several x-ray PTP1B-inhibitor complex structures have been reported (24–26). However, to our surprise and for reasons that will be discussed below, we were not able to obtain any crystals of TC-PTP complexed with any of these inhibitors. Therefore, we turned our attention to the uncomplexed version and obtained the apo structure.

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**TABLE II**

Kinetic constants for the hydrolysis of p-nitrophenyl phosphate at pH 6.5 and 25 °C

| Enzyme | $k_{cat}$ | $K_m$ | $k_{cat}/K_m \times 10^{-2}$ |
|--------|----------|-------|----------------------------|
| PTP1B  | 51.2 ± 2.0 | 0.47 ± 0.02 | 190.5 |
| TC PTP | 53.9 ± 1.8 | 0.48 ± 0.03 | 113.2 |

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**FIG. 1.** PTP1B and TC-PTP alignment. Identical residues are typed in green, similar residues are in blue, and different residues are in red. The full-length sequences for both proteins are used.
bonds to the amine of Gln-264. As indicated, a number of van der Waals contacts stabilize the complex formation as follows. (i) the Cβ atom of Thr-129 interacts with the Cβ and Cγ side chain atoms of Asp-50; (ii) the Glu-133 Cγ atom interacts with the Cβ atom of Phe-183; (iii) Leu-135 interacts with Phe-183; (iv) Leu-145 interacts with Tyr-48; (v) Leu-146 interacts with Val-121; (vi) Val-150 interacts with the Cγ and Cβ atoms of both Arg-43 and Arg-49; and finally (vii) Leu-158 is in van der Waals contact with the Cβ of Ser-120. Furthermore, the side chain hydroxyl group of Ser-147 is in hydrogen bond contact with the backbone carbonyl group of Ser-120, and Glu-148 hydrogen bonds to the backbone nitrogen of Arg-49 as well as to a water molecule that further hydrogen bonds to the backbone nitrogens of both Arg-49 and Asp-50. Finally, the side chains of Thr-155 and His-176 (via a water molecule) hydrogen bond with the guanidinium group of Arg-49.

Phosphate Mimic by an Aspartic Acid and Two Molecules—As described, Asp-131 is positioned into the active site cleft of TC-PTP. However, the carboxylic acid group is not in direct contact with the P loop as previously seen for charged ligands in the active site pocket of PTPs (24–26, 34–36). Instead, two water molecules are located between the carboxyl group of Asp-131 and the P loop mediating the charge and hydrogen bonds. These two water molecules are in a position to make hydrogen bonds to the backbone nitrogens of Ile-220, Gly-221, and Arg-222. Furthermore, the distance to the sulfur atom of Cys-216 from one of the water molecules is 3.0 Å. When the TC-PTP structure is superimposed on the PTP1B(C215S) phosphorylated Tyr (pTyr) structure (Protein Data Bank code 1PTV), it is apparent that the above water molecules mimic the hydrogen-bonding function of two of the three phosphate oxygen atoms (see Fig. 5c). The water molecules and the phosphate oxygen atoms are not exactly superimposed (the superimposition was performed on all protein atoms between TC-PTP and PTP1B). However, small displacements are also observed in the position of the P loop of TC-PTP and PTP1B. In addition to coordinating the position of the two water molecules Asp-131 is involved in a direct hydrogen bond to the amide of Gln-260. The Gln-260 side chain is stretched into (or toward) the carboxylic acid group of Asp-131, in contrast to both structures of PTP1B (i.e. in the apo or substrate/inhibitor form, see Fig. 5, a and c). Gln-260 in TC-PTP corresponds to the highly conserved Gln-262 in PTP1B, a residue that is critical in the second step of substrate hydrolysis (37).

Differences in the Proximity of the Active Site Pocket—We have recently used low resolution homology modeling, the so-called Ca variation score to identify three-dimensionally conserved and non-conserved surface areas on PTPs (3). Furthermore, the Ca variation score analysis in conjunction with primary sequence analysis allowed the identification of unique combinations of amino acid residues that might be addressed in structure-based design of selective inhibitors. The present study with experimentally determined x-ray structures of PTP1B and TC-PTP allows a direct comparison of these enzymes at the atomic level. We have in particular focused our attention on areas in proximity of the active site pocket, i.e. areas that might be simultaneously addressed by active site-directed inhibitors. As illustrated in Fig. 6, the surfaces of the two enzymes are very similar. However, two areas stand out as different and, hence, regions that potentially (i) could confer different substrate recognition capacity onto the two PTPs and (ii) might be used for the design of selective inhibitors for each of the enzymes. One of these areas is defined by the following residues and is found at the distal part of a region that we have termed the 258/259' gateway (24) (TC-PTP residues are in italics, and PTP1B residues are in bold): His-34/Cys-32, Glu-

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**The TC-PTP Structure**

Residues 5–277 of TC-PTP were identified and built into the electron density maps. The final structure contains two disordered loops, 117–121 and 236–242, with only backbone atoms traceable in the 2F_β – F, electron density maps. Cys-94 and Cys-95 both contained covalently bound β-mercaptoethanol (used as a reducing agent during purification and crystallization). Forty water molecules were inserted during refinements.

The WPD loop of TC-PTP was found in an open conformation. Thus, all structural comparisons with PTP1B are based on the apo form of PTP1B (Protein Data Bank code 2HNP). Overall, no differences were identified in the secondary or tertiary structures between TC-PTP and PTP1B. Minor differences are observed in loop areas between the two structures (see Fig. 3). The root mean square deviation between the TC-PTP and PTP1B structure (for all equivalent atoms) is 1.82 Å (calculated using Quanta).

**Crystal Packing—**Our lack of success in co-crystallizing TC-PTP with inhibitors is most likely explained by unusual crystal packing along the 2 axis (space group P4_2_2_2) involving the active site pocket and surrounding residues. A loop corresponding to residues 130–132, the “DDQ loop,” from one molecule was inserted in the active site of a neighboring molecule, resulting in a continuous row of TC-PTP molecules (Fig. 4a). The active site blockage is not limited to the DDQ loop but involves a total surface area of 1183 Å^2, resulting in a continuous row of TC-PTP molecules (Fig. 4). Thus, all structural comparisons with PTP1B are based on the apo form of PTP1B (Protein Data Bank code 2HNP). Over-all, no differences were identified in the secondary or tertiary structures between TC-PTP and PTP1B. Minor differences are observed in loop areas between the two structures (see Fig. 3). The root mean square deviation between the TC-PTP and PTP1B structure (for all equivalent atoms) is 1.82 Å (calculated using Quanta).

**Active Site Interface Interactions—**In the following, the residues that belong to the patch that is on the blocking (or inhibiting) molecule are denoted “inhibitor residues” (in italics), whereas the residues that belong to the active site patch of the neighboring molecule are described as “TC-PTP residues” (in bold).

Both of the carboxylic acid groups of Asp-130 and Asp-131 from the DDQ loop hydrogen bonds to the amine side chain group of Gln-264. Furthermore, the side chain of Asp-131 interacts with the P loop via two hydrogen-bonding water molecules (see details below). The amine of Gln-132 hydrogen

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**Fig. 2. Chemical structures.**
Lys-39 and Tyr-54 are a cluster of three residues accessible from the active site pocket. The other area of difference is a cluster of three residues, Gln-19/Ala-17, Leu-23/Glu-21, and Pro-262/Ala-264, which are directly accessible when the WPD loop is in the open conformation and with access over the aromatic Phe-183/Phe-182 when in a closed conformation.

Conformation of the Lys-122 Loop—In contrast to the reported structures of other PTPs (science.novonordisk.com/ptp), the highly conserved Lys-122 (corresponding to Lys-120 in PTP1B) is found in an open conformation. In other PTPs the equivalent lysine residues point toward the active site pocket and participate in defining boundaries of the active site pocket. Although side chain movements of Lys-122 (TC-PTP numbering) have been observed in many of the reported PTP1B structures in complex with various ligands and substrates, backbone movements have to our knowledge not been described previously. Indeed, superimposition of all the vertebrate PTP structures shows that the loop containing the lysine residues equivalent to Lys-122 in TC-PTP (the “Lys-122 loop”) only exhibit side chain variations and not backbone differences. Therefore, the Lys-122 loop has been regarded as structurally stable, with flexibility limited to the side chain only. As shown in Fig. 7, Lys-122 is forced (by crystal packing interactions described above) into a conformation pointing away from the active site pocket, leaving the active site open and not as the normally well defined pocket. Lys-122 moves 6 Å, measured at backbone level between the PTP1B and TC-PTP structures. Of note, the electron density map indicates significant flexibility of the Lys-122 loop when found in the open conformation. The Lys-122 loop is defined by residues 116–123 and, thus, includes the invariant Glu-117, which normally forms a salt bridge with Arg-222 in the P loop (38, 39). This salt bridge, seen in all other PTP structures, anchors the Lys-122 loop and defines the architecture and function of the P loop. In the TC-PTP structure reported here, there is no similar salt bridge, and as a result, Glu-117 points into the solvent with the carboxyl group more than 10 Å away from its normal position. Despite this, only a minor shift in the P loop and a limited rotation of the guanidinium group of Arg-222 are observed as the differences between the P loops of PTP1B (2HNP) and the TC-PTP structure reported here. Therefore, although the movement of the Lys-122 loop and Glu-117 is clearly the result of crystal packing and probably does not reflect the normal structure of TC-PTP, it is tempting to speculate (i) that the importance of the Glu-117–Arg-222 salt bridge in stabilizing the P loop may have been overestimated (or can be compensated for by ligand or substrate binding) and (ii) that such dramatic movement may also be induced by high affinity, low molecular weight inhibitors and, hence, can potentially be used for the design of selective PTP inhibitors.

**DISCUSSION**

PTP1B was the first protein-tyrosine phosphatase to be isolated and characterized as described in two landmark publications (5, 6). This was soon followed by hectic cloning efforts leading to the isolation of cDNAs encoding PTP1B, TC-PTP, and a number of other PTPs. Although detailed enzyme kinetic, mutational, and structural analyses of the bacterial *Yersinia* PTP over the years has provided invaluable information on PTP function (40–43), PTP1B has remained a favorite among researchers in the field. Numerous reports have provided significant insight into the function of PTPs using PTP1B as a model enzyme. Surprisingly, from a structural point of view, the highly homologous TC-PTP has received much less attention. Thus, despite the fact that TC-PTP was cloned more than a decade ago, the x-ray structure has not yet been reported. Because PTP1B is now considered an attractive drug target for treatment of type 2 diabetes (23), we decided to perform a detailed functional and structural comparison of these enzymes. In particular, we wanted to investigate if structural studies would provide evidence that selective, low molecular weight inhibitors for either of the two enzymes could be designed.

Using structure-based design strategies based on a combined approach involving detailed enzyme kinetic analyses with a set of wild type and mutant PTPs in combination with protein x-ray crystallography and modeling, we have previously been able to develop highly selective PTP1B inhibitors. Importantly, each step in the optimization process was based on efficient medium to high throughput x-ray crystallography of PTP1B 1–321 complexed with appropriate ligands. Typically, the turnaround time from novel compound to novel x-ray structure was less than a week. Therefore, it came as a surprise that we were not able to crystallize TC-PTP in complex with inhibitors that had also been shown to be efficient TC-PTP inhibitors. As described above, the lack of success in these co-crystallization studies seems to be due to a multimerization process, where residues 130–132, the DDQ loop, from one molecule are inserted into the active site of the neighboring molecule, resulting in a continuous string of interacting TC-PTP molecules.

PTP1B has previously been crystallized in six different space groups (see Table IV). In all of these space groups, the crystal-

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**Table III**

| Enzymes  | Compound 1  | Compound 2  | Compound 3  | Compound 4  |
|----------|-------------|-------------|-------------|-------------|
| PTP1B    | 34.0 ± 1.1  | 7.3 ± 0.6   | 19.3 ± 0.6  | 1.01 ± 0.04 |
| TC PTP   | 39.8 ± 1.6  | 7.5 ± 0.5   | 20.2 ± 0.6  | 1.47 ± 0.21 |

**Fig. 3. Co trace of TC-PTP and PTP1B.** TC-PTP is colored in light green, and PTP1B is in yellow. The structures were superimposed using Quanta.
packing arrangements for PTP1B have never been with a direct blockage of the active site pocket and do not utilize the residues 129–148, as seen in the current TC-PTP crystal packing. Of note, the crystal packing for TC-PTP observed here has never been reported for PTP1B. This difference is most likely due to the differences in the DDQ loop (i.e. in one of the four clusters of sequence differences between the two enzymes), which in PTP1B is EEK, i.e. all three residues are larger than the corresponding residues in TC-PTP, and the lysine residue, especially, will prohibit an interaction in PTP1B as seen for TC-PTP.

Previously, protein x-ray crystallography has provided evidence that the activity of the receptor-like PTPα might be negatively regulated by homodimerization (44). When the membrane-proximal domain of PTPα (apo structure) was crystallized, a symmetric dimer was formed in which a so-called wedge from one molecule was inserted into and thereby blocked the active site of the other molecule and vice versa. The sequences of the wedge region are conserved in receptor-like PTPs, thus suggesting a common, general regulatory mechanism for receptor PTPs involving the described homodimerization (44–46) and perhaps also heterodimerization (47). It remains to be demonstrated how such dimerization processes are to be regulated in vivo. But intriguingly, it was recently demonstrated that an inactivating point mutation in the putative wedge of CD45, a receptor-like PTP that is required for positive signaling in T cells, leads to lymphoproliferation and autoimmunity in transgenic mice (48). Although no structural information has yet been reported for CD45, this suggests that homodimerization could play a critical role in controlling the activity of this important PTP and perhaps other PTPs. At present, we do not know if the above autoinhibition of TC-PTP is biologically relevant similar to that observed for receptor-like PTPs. Our enzyme kinetic evaluation with isolated PTP domains does not indicate this to be the case. Also, in contrast to the homodimerization of receptor-like PTPs, we observe multimerization. However, it is interesting that previous studies show that the C-terminal part of TC-PTP has a negative influence on enzyme activity. Thus, in the early studies by Zander and co-workers (49), it was found that removal of the hydrophobic C terminus of TC-PTP resulted in a 30-fold increase in activity (49). In addition, limited proteolysis of TC-PTP released a highly active 33-kDa fragment, which again could be inhibited by the addition of the non-catalytic C-terminal segment of the 45-kDa TC-PTP (50). This indicates that the autoinhibition is caused by intramolecular interactions, whereas our observations are consistent with intermolecular interactions. Could it be that our x-ray structure fortuitously reflects a novel in vivo autoinhibitory mechanism?

It is of interest that Shoelson and coworkers (51) recently
FIG. 5. The DDQ loop binding motif. a, binding of Asp-131 from the DDQ loop (from one TC-PTP molecule) and two water molecules to the active site pocket P loop and Gln-260 (of a neighboring molecule). The atoms are colored according to atom type: carbons are in light green (in dark green for the Asp-131 carbon atoms), oxygens are in red, and nitrogens are in blue. The distances for the marked possible hydrogen bonds are in Å. b, stereo picture of the DDQ loop, the two water molecules, Gln-260, and P loop together with the final 2Fo − Fc electron density map. The atoms are colored as described in a. The 2Fo − Fc electron density map is colored at one sigma level in blue and three sigma level in red. c, stereo picture of the PTP1B(C215S)-phosphotyrosine (pTyr) structure (Protein Data Bank code 1PTV) superimposed on the TC-PTP structure for comparison of the binding mode between the Asp-131 with water molecules and the natural substrate phosphotyrosine. TC-PTP atoms are colored in light green (Asp-131 in dark green), and PTP1B(C215S)-phosphotyrosine atoms are in yellow. The critical oxygen atoms are colored in red.
have demonstrated autoinhibition of SHP-2 by a mechanism that resembles that presently described for TC-PTP by the insertion of the DDQ loop into the active site. These authors demonstrated that the N-terminal SH2 domain directly blocked the enzyme active site by insertion of the so-called D/H/E loop deeply into the catalytic cleft. Of note, the side chains of Asp-61 (from the SH2 domain) and Cys-459 of the PTP active site are hydrogen-bonded through a conserved water molecule, and a network of hydrogen bonds involving a number of residues stabilizes the interaction of the D/H/E loop with the enzyme active site. Although the specific binding pattern is different in TC-PTP, it is noteworthy that an Asp also plays a significant and central role for binding in this case.

Our previous studies have demonstrated that it is possible to use structure-based design to develop highly selective inhibitors of PTP1B utilizing residue 48 (attraction-repulsion) and the 258–259 region (steric fit-steric hindrance). However, because these regions are almost identical in TC-PTP, it is likely that active site-directed inhibitors addressing these areas in PTP1B will inhibit TC-PTP with equal potency. In accordance with this, we have recently demonstrated that two pyran-based active site inhibitors showed similar potency against the two enzymes (24). These observations have been extended further in the present analysis, demonstrating that selectivity for either of these enzymes must be achieved by addressing other areas. It is of significant interest that despite the high sequence and structural identity of TC-PTP and PTP1B, we and others (34) have been able to identify areas close to the active site that are structurally different and which might be utilized in developing PTP1B- or TC-PTP-selective inhibitors in the future. We speculate that the intrinsic differences in substrate recognition discussed above may at least in part be attributed to these areas.

The overall purpose of the present study was to investigate if...
selective inhibitors could be expected to be developed against either of the two highly homologous PTPs, PTP1B or TC-PTP. In particular, we were interested in identifying areas close to the active site that show significant structural differences between these enzymes, thus potentially allowing optimization of active site-directed inhibitors into selective PTP1B inhibitors (versus TC-PTP) or TC-PTP inhibitors (versus PTP1B). Two areas of potential interest have been identified: (i) a region that we have termed the 258-259 gateway (24) (TC-PTP residues are in italics, and PTP1B residues are in bold), His-34/Cys-32, Glu-41/Lys-39, and Tyr-54/Phe-52, which seem to be directly accessible from the active site pocket, and (ii) a cluster of three residues, Gln-21/Ala-17, Leu-23/Cys-32, and Phe-52/Cys-32, that is highly conserved across the PTP superfamily. Upon immediate inspection, these differences may be considered too minor to obtain selectivity. However, it should be noted that subtle differences in the ATP binding sites of kinases have previously been used successfully to develop selective inhibitors (52). Also, a recent structural comparison of the closely related insulin-like growth factor 1 receptor kinase and the insulin receptor kinase (>80% identity) led to the identification of similar minor differences that the authors hypothesized could be used for development of selective ATP-competitive inhibitors of each kinase (53). Furthermore, using a combination of detailed enzyme kinetics, mutational analyses, and x-ray crystallography, we have previously demonstrated that significant selectivity can be obtained by addressing one single residue with sequence difference in the PTP family (26). Finally, the present study also indicates that the Lys-222 loop may be addressed in an unexpected manner by disrupting the conserved salt bridge between Glu-117 and Arg-222.

Although it remains to be demonstrated if these areas indeed can be used for structure-based design of selective PTP1B or TC-PTP inhibitors, we hope that the x-ray structure provided will serve as inspiration for future drug design efforts. Such selective inhibitors could be invaluable tools in determining the exact biological roles of these two highly conserved and closely related PTPs.

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