Structure of the Catalytic Domain of Protein Tyrosine Phosphatase Sigma in the Sulfenic Acid Form

Tae Jin Jeon^{1,3}, Pham Ngoc Chien^{1,3}, Ha-Jung Chun², and Seong Eon Ryu^{1,*}

Protein tyrosine phosphatase sigma (PTP σ) plays a vital role in neural development. The extracellular domain of PTP σ binds to various proteoglycans, which control the activity of 2 intracellular PTP domains (D1 and D2). To understand the regulatory mechanism of PTP σ , we carried out structural and biochemical analyses of PTP σ D1D2. In the crystal structure analysis of a mutant form of D1D2 of PTP σ , we unexpectedly found that the catalytic cysteine of D1 is oxidized to cysteine sulfenic acid, while that of D2 remained in its reduced form, suggesting that D1 is more sensitive to oxidation than D2. This finding contrasts previous observations on PTP α . The cysteine sulfenic acid of D1 was further confirmed by immunoblot and mass spectrometric analyses. The stabilization of the cysteine sulfenic acid in the active site of PTP suggests that the formation of cysteine sulfenic acid may function as a stable intermediate during the redox-regulation of PTPs.

INTRODUCTION

Protein-phosphorylation plays a pivotal role in cellular signal transduction, cell growth and differentiation, affecting cancer, diabetes, and neurological diseases (Blaskovich, 2009; Ganguly et al., 2012; Tonks, 2006). Protein tyrosine phosphatases (PTPs), which dephosphorylate phosphorylated proteins are classified into classical PTPs and dual-specificity phosphatases. Classical PTPs dephosphorylate phospho-tyrosine, whereas dual-specificity phosphatases can dephosphorylate both phospho-tyrosine and phospho-serine/threonine. Classical PTPs are further divided into transmembrane receptor-like PTPs and nonreceptor-like PTPs (Alonso et al., 2004). Transmembrane receptor-like PTPs (RPTPs) mediate cellular signaling through the membrane.

PTP sigma (PTP σ) is a RPTP that plays a central role in neural development (Wallace et al., 1999). PTP σ knockout mice exhibit neurological and neuroendocrine defects (Elchebly et al., 1999; Shen et al., 2009; Wallace et al., 1999). These mice have also an increased nerve regeneration phenotype (Coles et al.,

2011). The extracellular domain of PTP σ carries tandem repeats of immunoglobulin-like domains and fibronectin type III domains and binds to both chondroitin and heparin sulfate proteoglycans (Coles et al., 2011). The binding of chondroitin and heparin sulfate proteoglycans to PTP σ inhibits and promotes neural cell growth, respectively, by controlling the formation of PTP σ clusters.

PTP activity is regulated by intracellular reactive oxygen species that inhibit the activity through oxidation of the active site cysteine (Cys) (Tabernero et al., 2008). The first-step of oxidation of the Cys residue results in the formation of Cys-sulfenic acid that is reversed to the sulfhydryl form of Cys upon reaction with reducing agents (Tonks, 2005). However, sulfenic acid is unstable, and prone to further oxidation towards sulfinic and sulfonic acid forms, which are irreversible in most cases. To avoid the irreversible oxidation of the active-site Cys, sulfenic acid can be converted to more stable forms, including sulfenyl amides or disulfide bonds (Sivaramakrishnan et al., 2010; Tonks, 2005). However, sulfenyl amides have been found in only a couple of PTPs (Salmeen et al., 2003; van Montfort et al., 2003; Yang et al., 2007), and the disulfide bond requires 1 or 2 additional Cys residues near the active-site Cys. Thus, it is likely that there are other mechanisms for the redox regulation and intermediate formation in various PTPs.

The cytoplasmic region of PTP σ contains 2 PTP catalytic domains, D1 and D2, arranged in a tandem fashion. Both D1 and D2 are classical PTP-type domains and contain a conserved CX₅R catalytic loop (Siu et al., 2007). However, D2 lacks catalytic activity and plays a regulatory role in the PTP σ -mediated signaling (Wallace et al., 1999). All catalytically active PTP domains contain the intact WPD loop with the tryptophan (Trp)-proline (Pro)-aspartate (Asp) sequence. The lack of the catalytic activity in D2 is mostly due to the replacement of Asp in the WPD loop by glutamate (Glu). Previously, the active-site Cys of D2 in PTP α was shown to be more sensitive to oxidation than that of D1 (Groen et al., 2008; Persson et al., 2004). This observation led to the hypothesis that oxidized D2 may trigger conformational changes in the cytoplasmic D1D2 assembly leading to the regulation of D1 activity.

¹Department of Bioengineering, College of Engineering, ²Department of Radiation Oncology, College of Medicine, Hanyang University, Seoul 133-070, Korea, ³These authors contributed equally to this work.

*Correspondence: ryuse@hanyang.ac.kr

Received January 30, 2013; revised April 15, 2013; accepted April 18, 2013; published online May 30, 2013

Keywords: crystal structure, protein tyrosine phosphatase sigma, proteoglycan, redox regulation, sulfenic acid

To characterize the redox regulation of PTP σ , we generated a mutant form of PTP σ D1D2 and performed structural and biochemical analyses. Unexpectedly, the structure determination revealed that the catalytic Cys of D1 is oxidized to Cys-sulfenic acid, whereas that of D2 remained in its reduced form. The formation of Cys-sulfenic acid of D1 was further demonstrated by immunoblot and mass spectrometric analyses. In contrast to previous results on PTP α , these observations indicate that Cys of D1 in PTP σ is more sensitive to oxidation than that of D2. The observation of Cys-sulfenic acid in the crystal structure of PTP σ indicates that it is relatively stable and does not undergo further oxidation to irreversible variants. The findings of this study provide evidence for Cys-sulfenic acid as a stable redox intermediate in the regulation of PTP activity during oxidative signaling.

MATERIALS AND METHODS

Expression and purification of the catalytic domains of PTP σ

The gene for the 2 intracellular catalytic domains (D1D2) of PTP σ corresponding to residues 1367-1948 was cloned between the *Bam*HI and *Eco*RI restriction sites of the expression vector pET28a. Seven Cys residues (at positions 1530, 1577, 1958, 1651, 1704, 1723, and 1932) were replaced with alanine (Ala) residues. We designated the resulting protein as 7CA-D1D2. For the immunoblot experiments, we generated 7CA-D1D2 with the C1589S mutation (7CA-D1D2-C1589S). The mutations were produced by using the QuickChange site directed mutagenesis kit (Stratagene) and confirmed by DNA sequencing. The *Escherichia coli* BL21 (DH3 α) cells were transformed with pET28a-7CA-D1D2. Protein overexpression was induced by addition of 0.2 mM isopropyl- β -D-thiogalactopyranoside at 18°C. The harvested cell pellets were lysed, and the protein was purified by using a Ni-nitriloacetate agarose column. The histidine-tag was removed by thrombin cleavage, and the protein was further purified by using a HiTrap Q anion exchange column and a gel filtration column (Sephacryl S-200). Fractions containing the protein were collected and concentrated to 20 mg/ml.

Crystallization and data collection

Crystallization was performed at 18°C using the hanging drop vapor diffusion method. Crystals were grown in drops containing 7CA-D1D2 (1.0 μ l) and the same volume of the reservoir solution containing 0.8 M succinic acid, pH 7.0. The diffraction data were collected with the beamline MX-I at the Pohang light source (PLS). The crystals were briefly soaked in a freezing buffer containing 20% glycerol before transferring them into the nitrogen stream. Diffraction data to 2.1 Å resolutions were indexed and integrated by using the program HKL2000 (Otwinowski and Minor, 1997). The crystals belonged to the P6 $_1$ space group with unit cell parameters of $a = 94.67$ Å, $b = 94.67$ Å, $c = 123.42$ Å, $\alpha = 90.0^\circ$, $\beta = 90.0^\circ$ and $\gamma = 120.0^\circ$.

Structure determination and refinement

The crystal structure of 7CA-D1D2 was determined with the molecular replacement method using the program Phaser in the CCP4i program suite (Winn et al., 2011). The structure of the wild type PTP σ -D1D2 (PDB code: 2F7H) was used for the search model. The structure of 7CA-D1D2 was refined by using the program Refmac5 (Winn et al., 2011) and rebuildings were done with the program WinCoot (Emsley et al., 2010). The 5.0% data were set aside for the R_{free} value estimation. After the initial

refinement, including 10 cycles of rigid body and restraint steps, the R_{cryst} and R_{free} values were 21.2% and 26.8%, respectively. After more rounds of refinement and rebuilding with the addition of water molecules, the final R_{cryst} and R_{free} values were 17.8% and 22.2%, respectively. There were no residues outside the allowed regions in the Ramachandran plot. The final structure coordinates have been deposited with RCSB Protein Data Bank (code: 4bpc).

Dimedone labeling and mass spectrometry

The 7CA-D1D2 (1 μ M) in a solution containing 20 mM HEPES, pH 7.0, 0.2 M NaCl, and 2 mM DTT was oxidized with H₂O₂ at different concentrations for 10 min at room temperature. An aqueous solution of 5,5-dimethyl-1,3-cyclohexanedione (Dimedone, Sigma Aldrich) was added to the oxidized samples to a final concentration of 10 mM and incubated for 1 h. Then, 0.1% RapiGest SF (Waters) and 0.5 mM DTT were added. To avoid further oxidation of the Cys residues, iodoacetamide was added to the samples to a final concentration of 15 mM and the mixtures were incubated for 30 min in the dark. For tryptic digestion, trypsin (Promega) was added to the sample in a 1:100 (w/w) enzyme/protein ratio, and the mixture was incubated overnight at 37°C. The peptide samples were desalted by using Zip-Tip (Millipore) and analyzed with a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (Shimadzu Biotech).

Immunoblot analysis

The 7CA-D1D2 and 7CA-D1D2-C1589S (10 μ M each) in a solution containing 20 mM HEPES, pH 7.0, 0.2 M NaCl and 2 mM DTT were oxidized with H₂O₂ at different concentrations for 10 min at room temperature. The oxidized samples were labeled with 5 mM dimedone (final concentration) in distilled water. The oxidized and dimedone-labeled protein samples were immediately subjected to 13% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The immediate gel loading prevents further oxidation of the samples. The protein bands in the gel were transferred to a polyvinylidene fluoride membrane (Bio-Rad). The membrane was blocked with 5% skim milk overnight at 4°C. Then, a dimedone-specific primary antibody (Millipore) was added, and the membrane was incubated for 3 h at room temperature. The membrane was washed 5 times with TBST buffer (1 $\times$) and then incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG (Abclon) for 1 h at room temperature. After washing 5 times with TBST, the membrane was incubated for 1 min in ECL solution and the protein band was visualized by using ChemiDoc XRS+ (Bio-Rad).

RESULTS

Structure determination

PTP σ D1D2 (residues 1,369-1,948) contains 11 Cys residues (at positions 1,501, 1,530, 1,577, 1,589, 1,958, 1,651, 1,704, 1,723, 1,790, 1,880, and 1,932). Among these, Cys1589 and Cys1880 are catalytic Cys residues located in the first and second PTP domains (D1 and D2), respectively. To avoid random oxidation, we mutated 7 Cys residues (1,530, 1,577, 1,958, 1,651, 1,704, 1,723 and 1,932) to Ala residues (7CA-D1D2). The catalytic Cys residues were left intact. Cys1501 and Cys1790, which are relatively close to the D1 and D2 catalytic Cys residues, respectively, remained also intact in order not to disrupt active site conformation. The catalytic activity of 7CA-D1D2 was identical to that of the native form (data not shown).

The crystallization of 7CA-D1D2 yielded high quality crystals

Table 1. Data and refinement statistics

Data	
Space group	$P6_1$
Unit-cell parameters	$a = b = 94.67 \text{ \AA}$, $c = 123.42 \text{ \AA}$ $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$
Resolution ($\text{\AA}$)	2.10 (2.14–2.10)
Reflections (total/unique)	205,759 / 36,574
Completeness (%)	98.15 (95.4) ¹
R_{merge} (%) ²	5.6 (32.9)
$I/\sigma I$	7.9 (7.4)
Refinement	
No. atoms	4773
Water molecules	227
$R_{\text{cryst}}/R_{\text{free}}$ (%)	17.8/22.2
Rms deviations	
Bond distances ($\text{\AA}$)	0.0173
Bond angles ($^\circ$)	1.7358
Average B factors ($\text{\AA}^2$)	51.252

¹The values in parentheses are for the highest resolution shell.

² $R_{\text{merge}} = \sum_j \sum_i |I_{hkl,j} - \langle I_{hkl} \rangle| / \sum_j \sum_i I_{hkl,j}$, where I is the intensity for the j^{th} measurement of an equivalent reflection with the indices h, k, l .

showing good diffraction and mechanical rigidity. The Cys mutations likely suppressed uncontrolled oxidations of the native protein that could harm the regular packing of the protein. The crystal diffracted beyond 1.8 $\text{\AA}$. We processed the diffraction data to a 2.1 $\text{\AA}$ resolution because of the weakening of the diffraction in several high-resolution regions. The structure of 7CA-D1D2 was determined by employing the molecular replacement method with the native PTP σ D1D2 structure as the target. Data collection and refinement statistics are shown in Table 1.

Structure comparison

The structure of 7CA-D1D2 reveals tightly associated D1 and D2 domains, each one carrying the active site pocket (Fig. 1). The overall structure of the mutated protein is almost identical to that of the native protein (PDB code: 2FH7) (Almo et al., 2007), except for the Cys-to-Ala mutations. The mutated residues show clear density for Ala residues. It appears that the mutations did not affect the overall structure of D1D2. The crystal contact of the 7CA-D1D2 structure does not indicate the wedge-mediated dimerization interaction found in PTP α (Bilwes et al., 1996; Jiang et al., 1999); purified 7CA-D1D2 exists as monomer in solution.

Despite the overall similarity, the new structure displayed much improvement in the flexible regions that were not clearly defined in the wild-type PTP σ D1D2 structure (Almo et al., 2007). For example, we could define side chain conformations of residues Arg1384, Lys1417, Asp1581, Val1656, and Val1825 that were represented as alternative conformations in the previous structure. We also built the side chains of residues Val1730, Val1731, Phe1870, Gln1872 and Asp1873 that were previously missing.

Sulfenic acid in the active site

In the initial rigid body refinement cycles with the molecular replacement solution of 7CA-D1D2, we found an extra density that extended from the sulfur atom of the active site Cys of D1. As the refinement proceeded, the extra density became clearer, and was modeled as an oxygen atom in the Cys-sulfenic acid (Fig. 2). In comparison to the Cys-sulfenic acid in the active site of D1, the active-site Cys of D2 showed a clear density for the sulfhydryl group (Fig. 2).

The crystal was not treated with an oxidizing agent. Thus, the oxidation of the active-site Cys of D1 was likely due to air oxidation. In the refined structure, there was 1 more circular density near the oxygen atom of the sulfenic acid. When we tried to model that density as further oxidized forms such as sulfinic or sulfonic acid, the resulting electron density did not fit the model in that region. Thus, we modeled the density as a water molecule (WAT in Fig. 2A). There is no such water molecule in the D2 Cys region, indicating that the water molecule in D1 may play a role in the stabilization of the sulfenic acid.

Fig. 1. Overall structure. The overall structure of 7CA-D1D2 was presented with labeling of Cys positions. (A) Ribbon diagram. The side chains of Cys residues including the Cys-sulfenic acid in the D1 active-site Cys (CSO1589) were represented as sticks. Cys residues other than CSO1589 include the D2 active-site Cys (Cys1880) and 2 other Cys residues (Cys1501 and Cys1790) near the active sites. (B) C α -trace diagram. The side chains of 7 Cys-to-Ala mutations were drawn together with the Cys side chains. In both figures, the N and C-termini were labeled.

Fig. 2. Electron density map in the active site. The 2Fo-Fc map was superposed with the refined model of the active-site Cys (CSO1589 and Cys1880) in the D1 and D2 domains, respectively. (A) His 1588-CSO1589-Ser1590 of the D1 domain is shown. CSO1589 is the sulfenic acid form of Cys1589. A water molecule (WAT) is located near the oxygen atom of CSO1589. (B) His1879-Cys1880-Ser1881 of the D2 domain is shown.

Fig. 3. Comparison of the active site structures. The structures of the D1 and D2 active sites of similar orientations were presented as ribbon models with side chains of key residues (See the text). (A) Active site of the D1 domain. (B) Active site of the D2 domain. The distance between the OE2 atom of Glu1928 and the SG atom of Cys1880 is indicated in the figure.

We then compared the environment of the 2 active sites in 7CA-D1D2 to understand the differential oxidation of the D1 Cys residue. Surprisingly, the neighboring residues were almost identical. The P-loop sequences (HCSAGVGRTG; residues 1588-1597 and 1879-1888 in D1 and D2, respectively) had the same orientation in both D1 and D2. The only difference near the Cys was the Gln1637 residue in a loop of D1 that was replaced by Glu1928 in D2 (Fig. 3). The negative charge of Glu1928 may inhibit the ionization of the active-site Cys and reduce the rate of reaction with reactive oxygen species, which would explain the lack of oxidation of the active site Cys of D2. The distance between the OE2 atom of Glu1928 and the SG atom of Cys1880 is 6.75 Å (Fig. 3), which is well within the long range electrostatic interaction distances of 9-12 Å (Piana et al., 2012).

Another difference between the 2 domains is in the WPD loop. The Asp residue of the WPD loop in D1 is changed to Glu in D2 resulting in a Trp-Pro-Glu (WPE). The active sites of both D1 and D2 are in the open state, and the WPD loop is distant from the active-site Cys. However, the WPD loop is expected to be flexible and is likely to get close to the active site in solution. Thus, the amino acid difference in the flexible WPD loop may affect oxidation rate and stabilization of the active site Cys.

Sulfenic acid-specific labeling in solution

To prove sulfenic acid formation in D1, we performed further biochemical analyses including mass spectrometry and immunoblotting. For these analyses, we used the dimedone-labeling method in which the putative sulfenic acid is selectively labeled with dimedone (Crump et al., 2012; Klomsiri et al., 2010). For the mass-spectrometric analysis, we treated the purified 7CA-D1D2 with different concentrations of H_2O_2 and the oxidized protein samples were labeled with dimedone. Then, the oxi-

dized and dimedone-labeled samples were subjected to trypsin digestion and mass-spectrometric analysis. The mass spectra indicated that the peak of the dimedone-labeled D1 active-site peptide (TANPPDAGPIVH-C [dimedone] -SAGVGR, residues 1576-1595, including the active-site Cys1589) increases with increasing H_2O_2 concentration (Fig. 4). The D1 active-site peptide has a mass of 1917.96 Da and labeling with dimedone results in a mass increase of 138.18 Da. Thus, the expected MH^+ peak of the dimedone-labeled peptide is 2,057.14 m/z corresponding to the leftmost peak of the group of peaks at around 2,057 m/z in Fig. 4.

In comparison to the increase in peak intensity of the dimedone-labeled D1 active site peptide, there was no change in the intensity of the peaks around 2,071 m/z whose leftmost peak corresponded to the MH^+ peak of another D1 peptide (AYIATQGPLAETTEDFWR; residues 1749-1766; 2,068.99 Da). In addition, there were only weak peaks for the putatively dimedone-labeled D2 active-site peptide (EQFGQDGPISVH-C [dimedone] -SAGVGR; residues 1868-1886; native mass: 1943.90 Da; dimedone-labeled mass: 2082.08 Da) whose MH^+ peak was expected to occur at around 2,083 m/z (Fig. 4). These results indicate that the Cys of D1 has a higher tendency for oxidation than that of D2, and the D1 Cys-sulfenic acid is stable enough to be detected by dimedone labeling.

The Cys-sulfenic acid form of 7CA-D1D2 was also confirmed by immunoblot analysis. The purified 7CA-D1D2 protein was treated with different concentrations of H_2O_2 , and the resulting oxidized proteins were labeled with dimedone. The oxidized and dimedone-labeled 7CA-D1D2 was loaded onto an acrylamide gel and transferred to a polyvinylidene fluoride membrane. The dimedone-labeled protein was probed by the anti-dimedone primary antibody (Fig. 5). In the gel, the H_2O_2 concentration-dependent increase in the band of dimedone-labeled 7CA-

Fig. 4. MALDI-TOF mass spectrometry analysis. Tryptic fragments of the oxidized and dimedone-labeled 7CA-D1D2 proteins were analyzed by the MALDI-TOF mass spectrometry. (A), (B), and (C) are mass spectra of the 7CA-D1D2 proteins oxidized with 2.0, 1.0 and 0.0 mM H_2O_2 , respectively.

Fig. 5. Immunoblot analysis. The oxidized and dimedone-labeled 7CA-D1D2 and 7CA-D1D2-C1589S were probed by a dimedone-specific antibody. The protein samples of 10 μ M each were treated with 0.1–2.0 mM H_2O_2 prior to dimedone labeling. Lanes 1–4 and 5–8 are 0.1, 0.4, 1.0 and 2.0 mM H_2O_2 -treated 7CA-D1D2 and 7CA-D1D2-C1589S, respectively. The upper panel is the result of immunoblot and the lower panel is the Coomassie Blue-staining result of the stripped membrane that was used in the immunoblot.

D1D2 (64 kDa) was clearly observed, revealing the existence of the sulfenic acid in 7CA-D1D2. We also carried out the same experiment with the mutant (7CA-D1D2-C1589S) protein to confirm the relative sensitivity of the D1 Cys over the D2 Cys. In lanes 5–8 of Fig. 5, the 7CA-D1D2-C1589S mutant protein shows a very low level of dimedone labeling and the H_2O_2 concentration-dependent increase in the band of dimedone labeled mutant protein does not appear. In comparison to the immunoblotted bands (upper panel of Fig. 5), the Coomassie Blue-stained bands (lower panel) show equal densities. Thus, sulfenic acid formation appears to be specific for the D1 Cys.

DISCUSSION

The redox-mediated activity regulation plays a critical role in the function of PTPs (Tonks, 2005). High intracellular levels of reactive oxygen species inhibit the enzymatic activity of PTPs by oxidizing the active-site Cys residue. In the current study, crystal structure analysis of the catalytic domains (D1D2) of PTP σ revealed the formation of Cys-sulfenic acid in D1. Mass spectrometry and immunoblot analyses also proved the existence of sulfenic acid. In contrast, the Cys residue of D2 showed no indication of Cys-sulfenic acid formation.

The presence of Cys-sulfenic acid in the active site of PTPs has been regarded as an unstable state that has to be protected by sulfenyl-amide or disulfide bond formations. However, sulfenyl-amide was found in only 2 cases, i.e., PTP1B and the D2 domain of PTP α (Salmeen et al., 2003; van Montfort et al., 2003; Yang et al., 2007), indicating that it may not be a general mechanism for the PTP redox regulation. With regard to the mechanism of disulfide bond formation, there are many PTPs that do not have additional Cys residues near the active-site Cys. Thus, it is likely that other PTP redox regulation mechanisms exist, other than sulfenyl-amide or disulfide formations. The current results suggest that the stabilized Cys-sulfenic acid can be one of the other mechanisms for the reversible redox regulation of PTPs. Stabilized Cys-sulfenic acid has been found in peroxiredoxins and other redox-related proteins (Choi et al., 1998; Nakamura et al., 2008; Salsbury et al., 2008). This is the first time that the sulfenic acid form has been detected in the active site of a PTP.

Oxidation of redox-sensitive proteins often mediates the proteins' functional modulation through structural switches. Disulfide-bond-mediated structural and functional switches were found in various proteins, including the bacterial transcription factor OxyR, the *Drosophila* vision-signaling protein INAD, and the hypertension-control protein angiotensinogen (Lee et al., 2004; Liu et al., 2011; Zhou et al., 2010). In these proteins, the first step of oxidation likely involves the formation of Cys-sulfenic acid of the redox-sensitive Cys. Cys-sulfenic acid reacts with another Cys that is more distant than the usual disulfide bond length, triggering conformational changes in the proteins (Ryu, 2012).

The discovery of a stable sulfenic acid in PTP σ suggests that the formation of a stable sulfenic acid can be used to trigger functional switches in redox sensitive proteins. The addition of 1 oxygen atom to the Cys sulfur is not a large change for structural switches. However, in a tightly packed structure, such a change could trigger structural switches. Recently, Lyn kinase was found to be directly activated by the oxidation of Cys466 and no Cys residue was identified that could function as disulfide bond partner (Yoo et al., 2011). Cys404 of the yeast cell cycle regulator Swi6p also forms a sulfenic acid for the oxidation-mediated activation of the protein (Chiu et al., 2011). In such cases, the stable Cys-sulfenic acid may play a role in the structural and functional changes of proteins.

The susceptibility of the D1 active site of PTP σ to oxidation found in the current study is different from that of PTP α , where the active-site Cys of D2 was more susceptible to oxidation than that of D1. The findings on PTP α led to the hypothesis that D2 senses oxidative stresses and functions as a redox sensor. The oxidation of the D2 Cys may trigger conformational changes that could favor dimerization of the cytoplasmic domain of PTP α leading to D1 activity regulation (Persson et al., 2004). The inhibition of PTP activity *via* dimerization is mediated by the wedge region of PTP α (Bilwes et al., 1996; Jiang et al., 1999). However, the wedge was not found in the structure of 7CA-D1D2 of PTP σ as well as other RPTPs such as CD45 (Nam et al., 2005) and LAR (Nam et al., 1999), indicating that the D2-mediated redox regulatory mechanism in PTP α is not a general process. Instead, the D1D2 domains of various RPTPs appear to employ different activity regulation mechanisms.

In conclusion, the current results on the oxidation of the D1 Cys and the relative stability of the Cys-sulfenic acid in PTP σ provide novel information regarding the activity regulation of the double catalytic domains of RPTPs and the reversible redox regulation of PTPs. Because there are more than 110 members

of PTPs in human (Alonso et al., 2004), we can expect various mechanisms for their activity and redox regulations. The current data indicate that the stabilized sulfenic acid in the active site can be a novel mechanism for the reversible redox regulation of the PTP family. Our results suggest that D2 of PTP σ may not function as a more sensitive redox sensor than that of D1. However, it is still possible that the Cys residue of D2 forms an intra- or inter-molecular disulfide bond with Cys residues that were mutated in this study. A more comprehensive oxidation study using various Cys mutant proteins of PTP σ would be necessary to provide the full picture of the redox regulation of the enzyme.

ACKNOWLEDGMENTS

This work was supported by a biomedical technology development grant from the National Research Foundation of Korea.

REFERENCES

- Almo, S.C., Bonanno, J.B., Sauder, J.M., Emtage, S., D'Irenzo, T.P., Malashkevich, V., Wasserman, S.R., Swaminathan, S., Eswaramoorthy, S., Agarwal, R., et al. (2007). Structural genomics of protein phosphatases. *J. Struct. Funct. Genomics* 8, 121-140.
- Alonso, A., Sasin, J., Bottini, N., Friedberg, I., Osterman, A., Godzik, A., Hunter, T., Dixon, J., and Mustelin, T. (2004). Protein tyrosine phosphatases in the human genome. *Cell* 117, 699-711.
- Bilwes, A.M., den Hertog, J., Hunter, T., and Noel, J.P. (1996). Structural basis for inhibition of receptor protein-tyrosine phosphatase- α by dimerization. *Nature* 382, 555-559.
- Blaskovich, M.A. (2009). Drug discovery and protein tyrosine phosphatases. *Curr. Med. Chem.* 16, 2095-2176.
- Chiu, J., Tactacan, C.M., Tan, S.X., Lin, R.C., Wouters, M.A., and Dawes, I.W. (2011). Cell cycle sensing of oxidative stress in *Saccharomyces cerevisiae* by oxidation of a specific cysteine residue in the transcription factor Swi6p. *J. Biol. Chem.* 286, 5204-5214.
- Choi, H.J., Kang, S.W., Yang, C.H., Rhee, S.G., and Ryu, S.E. (1998). Crystal structure of a novel human peroxidase enzyme at 2.0 Å resolution. *Nat. Struct. Biol.* 5, 400-406.
- Coles, C.H., Shen, Y., Tenney, A.P., Siebold, C., Sutton, G.C., Lu, W., Gallagher, J.T., Jones, E.Y., Flanagan, J.G., and Aricescu, A.R. (2011). Proteoglycan-specific molecular switch for RPTP-sigma clustering and neuronal extension. *Science* 332, 484-488.
- Crumpp, K.E., Juneau, D.G., Poole, L.B., Haas, K.M., and Grayson, J.M. (2012). The reversible formation of cysteine sulfenic acid promotes B-cell activation and proliferation. *Eur. J. Immunol.* 42, 2152-2164.
- Elchebly, M., Wagner, J., Kennedy, T.E., Lancot, C., Michaliszyn, E., Iltis, A., Drouin, J., and Tremblay, M.L. (1999). Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma. *Nat. Genet.* 21, 330-333.
- Emsley, P., Lohkamp, B., Scott, W.G., and Cowtan, K. (2010). Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* 66, 486-501.
- Ganguly, A., Sasayama, D., and Cho, H.T. (2012). Regulation of the polarity of protein trafficking by phosphorylation. *Mol. Cells* 33, 423-430.
- Groen, A., Overvoorde, J., van der Wijk, T., and den Hertog, J. (2008). Redox regulation of dimerization of the receptor protein-tyrosine phosphatases RPTPalpha, LAR, RPTPmu and CD45. *FEBS J.* 275, 2597-2604.
- Jiang, G., den Hertog, J., Su, J., Noel, J., Sap, J., and Hunter, T. (1999). Dimerization inhibits the activity of receptor-like protein-tyrosine phosphatase- α . *Nature* 401, 606-610.
- Klomsiri, C., Nelson, K.J., Bechtold, E., Soito, L., Johnson, L.C., Lowther, W.T., Ryu, S.E., King, S.B., Furdul, C.M., and Poole, L.B. (2010). Use of dimedone-based chemical probes for sulfenic acid detection evaluation of conditions affecting probe incorporation into redox-sensitive proteins. *Methods Enzymol.* 473, 77-94.
- Lee, C., Lee, S.M., Mukhopadhyay, P., Kim, S.J., Lee, S.C., Ahn, W.S., Yu, M.H., Storz, G., and Ryu, S.E. (2004). Redox regula-

- tion of OxyR requires specific disulfide bond formation involving a rapid kinetic reaction path. *Nat. Struct. Mol. Biol.* **11**, 1179-1185.
- Liu, W., Wen, W., Wei, Z., Yu, J., Ye, F., Liu, C.H., Hardie, R.C., and Zhang, M. (2011). The INAD scaffold is a dynamic, redox-regulated modulator of signaling in the *Drosophila* eye. *Cell* **145**, 1088-1101.
- Nakamura, T., Yamamoto, T., Abe, M., Matsumura, H., Hagihara, Y., Goto, T., Yamaguchi, T., and Inoue, T. (2008). Oxidation of archaeal peroxiredoxin involves a hypervalent sulfur intermediate. *Proc. Natl. Acad. Sci. USA* **105**, 6238-6242.
- Nam, H.J., Poy, F., Krueger, N.X., Saito, H., and Frederick, C.A. (1999). Crystal structure of the tandem phosphatase domains of RPTP LAR. *Cell* **97**, 449-457.
- Nam, H.J., Poy, F., Saito, H., and Frederick, C.A. (2005). Structural basis for the function and regulation of the receptor protein tyrosine phosphatase CD45. *J. Exp. Med.* **201**, 441-452.
- Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. *Methods in Enzymology* Vol. 276, Macromolecular Crystallography, part A, C.W. Carter, Jr. and R.M. Sweet, eds. (Academic Press), pp. 307-326.
- Persson, C., Sjoblom, T., Groen, A., Kappert, K., Engstrom, U., Hellman, U., Heldin, C.H., den Hertog, J., and Ostman, A. (2004). Preferential oxidation of the second phosphatase domain of receptor-like PTP-alpha revealed by an antibody against oxidized protein tyrosine phosphatases. *Proc. Natl. Acad. Sci. USA* **101**, 1886-1891.
- Piana, S., Lindorff-Larsen, K., Dirks, R.M., Salmon, J.K., Dror, R.O., and Shaw, D.E. (2012). Evaluating the effects of cutoffs and treatment of long-range electrostatics in protein folding simulations. *PLoS One* **7**, e39918.
- Ryu, S.E. (2012). Structural mechanism of disulphide bond-mediated redox switches. *J. Biochem.* **151**, 579-588.
- Salmeen, A., Andersen, J.N., Myers, M.P., Meng, T.C., Hinks, J.A., Tonks, N.K., and Barford, D. (2003). Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* **423**, 769-773.
- Salsbury, F.R., Jr., Knutson, S.T., Poole, L.B., and Fetrow, J.S. (2008). Functional site profiling and electrostatic analysis of cysteines modifiable to cysteine sulfenic acid. *Protein Sci.* **17**, 299-312.
- Shen, Y., Tenney, A.P., Busch, S.A., Horn, K.P., Cuascut, F.X., Liu, K., He, Z., Silver, J., and Flanagan, J.G. (2009). PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. *Science* **326**, 592-596.
- Siu, R., Fladd, C., and Rotin, D. (2007). N-cadherin is an *in vivo* substrate for protein tyrosine phosphatase sigma (PTPsigma) and participates in PTPsigma-mediated inhibition of axon growth. *Mol. Cell. Biol.* **27**, 208-219.
- Sivaramakrishnan, S., Cummings, A.H., and Gates, K.S. (2010). Protection of a single-cysteine redox switch from oxidative destruction: on the functional role of sulphenyl amide formation in the redox-regulated enzyme PTP1B. *Bioorg. Med. Chem. Lett.* **20**, 444-447.
- Taberner, L., Aricescu, A.R., Jones, E.Y., and Szedlacsek, S.E. (2008). Protein tyrosine phosphatases: structure-function relationships. *FEBS J.* **275**, 867-882.
- Tonks, N.K. (2005). Redox redux: revisiting PTPs and the control of cell signaling. *Cell* **121**, 667-670.
- Tonks, N.K. (2006). Protein tyrosine phosphatases: from genes, to function, to disease. *Nat. Rev. Mol. Cell Biol.* **7**, 833-846.
- van Montfort, R.L., Congreve, M., Tisi, D., Carr, R., and Jhoti, H. (2003). Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. *Nature* **423**, 773-777.
- Wallace, M.J., Batt, J., Fladd, C.A., Henderson, J.T., Skarnes, W., and Rotin, D. (1999). Neuronal defects and posterior pituitary hypoplasia in mice lacking the receptor tyrosine phosphatase PTPsigma. *Nat. Genet.* **21**, 334-338.
- Winn, M.D., Ballard, C.C., Cowtan, K.D., Dodson, E.J., Emsley, P., Evans, P.R., Keegan, R.M., Krissinel, E.B., Leslie, A.G.W., McCoy, A., et al. (2011). Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 235-242.
- Yang, J., Groen, A., Lemeer, S., Jans, A., Slijper, M., Roe, S.M., den Hertog, J., and Barford, D. (2007). Reversible oxidation of the membrane distal domain of receptor PTPalpha is mediated by a cyclic sulfenamide. *Biochemistry* **46**, 709-719.
- Yoo, S.K., Starnes, T.W., Deng, Q., and Huttenlocher, A. (2011). Lyn is a redox sensor that mediates leukocyte wound attraction *in vivo*. *Nature* **480**, 109-112.
- Zhou, A., Carrell, R.W., Murphy, M.P., Wei, Z., Yan, Y., Stanley, P.L., Stein, P.E., Broughton Pipkin, F., and Read, R.J. (2010). A redox switch in angiotensinogen modulates angiotensin release. *Nature* **468**, 108-111.