Differential Oxidation of Protein-tyrosine Phosphatases*

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Oxidation is emerging as an important regulatory mechanism of protein-tyrosine phosphatases (PTPs). Here we report that PTPs are differentially oxidized, and we provide evidence for the underlying mechanism. The membrane-proximal RPTPα-D1 was catalytically active but not readily oxidized as assessed by immunoprobing with an antibody that recognized oxidized catalytic site cysteines in PTPs (oxPTPs). In contrast, the membrane-distal RPTPα-D2, a poor PTP, was readily oxidized. Oxidized catalytic site cysteines in PTP immunoprobing and mass spectrometry demonstrated that mutation of two residues in the Tyr(P) loop and the WPD loop that reverse catalytic activity of RPTPα-D1 and RPTPα-D2 also reversed oxidizability, suggesting that oxidizability and catalytic activity are coupled. However, catalytically active PTP1B and LAR-D1 were readily oxidized. Oxidizability was strongly dependent on pH, indicating that the microenvironment of the catalytic cysteine has an important role. Crystal structures of PTP domains demonstrated that the orientation of the absolutely conserved PTP loop arginine correlates with oxidizability of PTPs, and consistently, RPTPα-D1, with a similar conformation as RPTPα-D1, was not readily oxidized. In conclusion, PTPs are differentially oxidized at physiological pH and H₂O₂ concentrations, and the PTP loop arginine is an important determinant for susceptibility to oxidation.

Phosphorylation of proteins on tyrosine residues has an important role in many cellular processes like proliferation, differentiation, and migration. Tyrosine phosphorylation is mediated by the balanced action of protein-tyrosine kinases and protein-tyrosine phosphatases (PTPs)¹ (1). Not only protein-tyrosine kinases but also PTPs have important roles in diseases, including cancer and diabetes (2–5). The human genome encodes 103 cysteine-based PTP family members, of which 38 are strictly phosphotyrosine-specific (4, 5). These “classical” PTPs are subdivided into transmembrane, receptor PTPs (RPTPs) (21 genes) and intracellular, nonreceptor PTPs (17 genes). The cytoplasmic PTPs encode a single PTP domain, whereas most RPTPs contain two catalytic domains. The membrane-proximal domain (D1) contains most catalytic activity (6, 7), whereas the membrane-distal domain (D2) has a regulatory function (8). The PTPs, including the RPTP-D2s, are highly conserved in sequence and three-dimensional structure (9–11). Only two amino acid residues that are conserved in all active PTPs, but not in RPTP-D2s, are responsible for the lack of catalytic activity in RPTP-D2s, because mutation of these residues renders RPTP-D2s active (10, 12, 13).

Relatively little is known about the regulation of PTPs. Reversible oxidation of the absolutely conserved catalytic site cysteine that is essential for catalysis (14) is emerging as an important regulatory mechanism (15). The catalytic cysteines are susceptible to oxidation because of their low pKₐ (16, 17). Oxidation of cysteine residues to sulfenic acid is reversible, whereas further oxidation to sulfonic (doubly oxidized) or sulfonic (triply oxidized) forms is irreversible (18). Oxidized PTP1B shows a newly identified bond, termed cyclic sulfenamide, in which the sulfur of the cysteine is covalently linked to the main chain nitrogen of the neighboring serine (19, 20). Cyclic sulfenamide, like sulfenic acid, can be reduced by thiols, and importantly, it suppresses oxidation to sulfonic and sulfonic acids.

There are many physiological stimuli that induce reactive oxygen species (ROS) production. For instance, ROS are produced in response to stimuli like UV light, growth factors, and insulin, leading to inactivation of PTPs (21–26). ROS-induced inhibition of PTP activity may be essential for stimulus-induced signaling. For instance, platelet-derived growth factor induces oxidation of Shp2, and N-acetyl cysteine, a ROS quencher, blocks Shp2 oxidation and reduces platelet-derived growth factor signaling (25). Interestingly, Reynolds et al. (27) demonstrated in a mathematical model that epidermal growth factor-induced epidermal growth factor receptor activation, together with ROS-mediated inactivation of PTPs is sufficient to cause lateral signal propagation.

PTPs are emerging as important redox sensors in cells. Recently, we found that RPTPα is regulated by oxidation in an unexpected way. Whereas RPTPα-D1 contains most of the catalytic activity, it appears that RPTPα-D2 is much more sensitive to oxidation than RPTPα-D1 in vitro and in cells in response to UV irradiation (28). Yet RPTPα is inactivated in
response to H$_2$O$_2$ treatment, because RPTP-$\alpha$-D2 undergoes a conformational change in response to oxidation, leading to stabilization of an inactive dimeric conformation (8, 29). Mutation of the catalytic cysteine in RPTP-$\alpha$-D2 renders RPTP-$\alpha$ less sensitive to oxidation, in that H$_2$O$_2$ and UV treatment of living cells do not lead to complete inactivation of RPTP-$\alpha$-C723S, whereas wild type RPTP-$\alpha$ is inactivated completely (8, 28).

In the present study, we investigated differential oxidation of PTPs. oxPTP immunoprobng and matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectrometry indicated that the mutations in the Tyr(P) loop and the WPD loop that are responsible for the difference in catalytic activity between RPTP-$\alpha$-D1 and RPTP-$\alpha$-D2 were also involved in the difference in oxidizability. Furthermore, we demonstrate that PTP1B and LAR-D1 were highly oxidizable. The susceptibility of PTPs to oxidation was strongly dependent on pH. Comparison of the crystal structures suggested that the orientation of the PTP loop arginine is important for the oxidizability of PTPs. Taken together, the subtle differences in the microenvironment of the catalytic cysteine determine the oxidizability of PTPs.

**MATERIALS AND METHODS**

**Constructs**—pGEX-based bacterial expression vectors encoding GST fusion proteins of RPTP-$\alpha$-D1 contained the region from amino acids 167 to 503 and RPTP-$\alpha$-D2 from amino acids 504 to 793 (30). D2-E690D, D2-V555Y, and D2-E690D/V555Y have been described (13). RPTP-$\alpha$-D1-Y262V and D1-D401E were made by site-directed mutagenesis and verified with pervanadate to convert reduced cysteines to the sulfonic acid form.

**Analysis of Oxidized PTPs Using the oxPTP Antibody**—Reversible oxidation was assayed using an antibody, oxPTP, that specifically recognizes the sulfonic acid form of catalytic site cysteines of PTPs (28, 32). As outlined in Fig. 1A, following treatment, the sample is alkylated to protect reduced cysteines. Subsequently, the sample is treated with dithiothreitol (DTT) to reduce reversibly oxidized cysteines and treated with pervanadate to convert reduced cysteines to the sulfonic acid form. Finally, the sulfonic acid form is detected using the oxPTP antibody by immunoblotting. The procedure has been described in detail (28). Briefly, GST fusion proteins, purified using standard procedures, were bound to glutathione beads. All of the incubations and washes were done with 20 mM Tris, pH 7.5, unless otherwise stated. The beads were washed twice with 20 mM Tris-HCl, pH 7.5, and then directly derivatized the unaffected cysteines. Finally, the beads were washed and incubated with 10 mM DTT for 10 min to reduce singly oxidized cysteines. The fusion protein was cleaved off by thrombin protease (4 h at room temperature), and the protein of interest was subjected to overnight tryptic digestion. For desalting, the peptides were adsorbed on C$_{18}$ ZipTips (Millipore) and washed with 0.1% trifluoroacetic acid. The peptides retained on the columns were eluted using a-cyano-4-hydroxy-cinnamic acid (10 mg/ml) in 10% acetonitrile, 0.1% trifluoroacetic acid. Peptide mass fingerprint spectra were recorded on a MALDI-ToF Voyager DE-STR (Applied Biosystems) mass spectrometer operated in positive ion Reflectron mode. After time-delayed extraction, the ions were accelerated to 20 kV for ToF mass spectrometric analysis. A total of 150 shots were acquired, and the signal was averaged per spectrum. MALDI-ToF spectra were analyzed using the Applied Biosystems Data Explorer software.

**RESULTS**

**Differential Oxidation of RPTP-$\alpha$-D1 and RPTP-$\alpha$-D2**—We investigated oxidation of PTPs using the oxPTP antibody (Fig. 1A). Consistent with a previous report (28), RPTP-$\alpha$-D1 was oxidized at 250 $\mu$M H$_2$O$_2$ but not at lower concentrations (Fig. 1B), and RPTP-$\alpha$-D2 was already detectably oxidized at the lowest concentration used (31 $\mu$M H$_2$O$_2$) (Fig. 1C). The negative control in the oxPTP experiments represents the fully reduced PTP, achieved by treatment with 10 mM DTT for 20 min. For the positive control, the alkylation step was omitted, leading to complete triple oxidation of the catalytic cysteine upon pervanadate treatment (Fig. 1).

In parallel with the oxPTP immunoprobng experiments, we performed PTP activity assays using p-nitrophenylphosphate as a substrate. RPTP-$\alpha$-D1 retained much of its activity at low concentrations of H$_2$O$_2$ (31 $\mu$M) (Fig. 1D). RPTP-$\alpha$-D2, although much less active than D1, is more susceptible to H$_2$O$_2$ than RPTP-$\alpha$-D1 and is already inactivated at low concentrations of H$_2$O$_2$ (31 $\mu$M; Fig. 1D). Together, these results show that RPTP-$\alpha$-D2 is more susceptible to oxidation than RPTP-$\alpha$-D1 as detected by the oxPTP antibody and by PTP activity assays.

**Two Residues Determine the Difference between RPTP-$\alpha$-D1 and RPTP-$\alpha$-D2**—Previously, we have shown that the difference in catalytic activity between RPTP-$\alpha$-D1 and RPTP-$\alpha$-D2 is caused by two residues in the Tyr(P) loop (Tyr$^{262}$ in D1 and Val$^{555}$ in D2) and WPD loop (Asp$^{401}$ in D1 and Glu$^{690}$ in D2) that are always conserved in catalytically active PTPs and never in inactive PTP-D2s. The single mutants in RPTP-$\alpha$-D2, E690D and V555Y, regain some of their activity, and the double mutant V555Y/E690D is as active as RPTP-$\alpha$-D1 (12, 13, 33). These mutants were tested for their susceptibility to H$_2$O$_2$ using the oxPTP antibody. Whereas wild type RPTP-$\alpha$-D2 was oxidized at 31 $\mu$M H$_2$O$_2$, RPTP-$\alpha$-D2-V555Y was only oxidized at 125 $\mu$M H$_2$O$_2$ and above (Fig. 2A). Even higher levels of H$_2$O$_2$ were required for RPTP-$\alpha$-D2-E690D oxidation (Fig. 2A). Moreover, RPTP-$\alpha$-D2-V555Y/E690D oxidation was only detected at 1 $\mu$M H$_2$O$_2$ (Fig. 2A).

To test whether the Tyr(P) loop and the WPD motif were responsible for low oxidizability of RPTP-$\alpha$-D1, Tyr$^{262}$ was mutated to valine, and Asp$^{401}$ was mutated to glutamate. RPTP-$\alpha$-D1-Y262V and RPTP-$\alpha$-D1-D401E were much more sensitive to oxidation than wild type RPTP-$\alpha$-D1, in that appreciable oxidation was already detected at 31–62 $\mu$M H$_2$O$_2$ (Fig. 2B), similar to wild type RPTP-$\alpha$-D2. These results support the hypothesis that the Tyr(P) loop and WPD loop are important for susceptibility to oxidation of RPTP-$\alpha$-D1 and RPTP-$\alpha$-D2.

We determined the catalytic activity of RPTP-$\alpha$-D1, RPTP-$\alpha$-D2, and their mutants, allowing direct comparison of PTP activity and oxidizability. Consistent with previous results (12, 13, 33), the single point mutations in RPTP-$\alpha$-D2 enhanced its
activity to some extent, whereas RPTPα-D2-V555Y/E690D exhibited catalytic activity similar to RPTPα-D1 (Fig. 2C). Mutation of Asp401 and Tyr262 completely abolished RPTPα-D1 activity (Fig. 2C), demonstrating that the Tyr(P) loop and WPD loop are essential for catalytic activity.

Mass spectrometry experiments were performed to further investigate oxidation of RPTPα-D1 and RPTPα-D2. Unfortunately, the Cys433 containing peptide of RPTPα-D1 did not resolve well in the MALDI-ToF spectra, and detection of Cys433 oxidation failed. The peptide containing Cys723 of RPTPα-D2 and its oxidized derivatives were readily detectable by MALDI-ToF (Fig. 3). At 1 mM H2O2, four peaks were evident in the spectra. As a result of the alkylation/reduction procedure, the reversibly oxidized Cys723 peptide was detected in its reduced form (m/z 2075.98), whereas alkylated Cys723 peptide corresponds to reduced Cys723 peptide (m/z 2133.00). The other two peaks, m/z 2107.98 and 2123.98, represent irreversibly oxidized Cys723 peptides, respectively. Reversibly oxidized Cys723 peptide was already detected at the lowest concentrations of H2O2 (31 μM), and the fraction of reversibly oxidized Cys723 peptide increased gradually with increasing H2O2 concentrations (Fig. 3). The sulfenic acid species were only detected at high H2O2 concentrations (250 μM H2O2 and higher) and sulfonic acid only at 1 mM H2O2.

Based on the MALDI-ToF spectra, we calculated the fraction of reversibly oxidized Cys723 peptide, relative to the total amount of Cys723 peptide. Wild type RPTPα-D2 was highly sensitive to oxidation, whereas RPTPα-D2-E690D was moder-
Differential Oxidation of PTPs

Fig. 3. MALDI-ToF analysis of oxidation of RPTPα-D2 and mutants. Purified RPTPα-D2 was treated with H2O2 and MALDI-ToF spectra were acquired as described under "Materials and Methods." The m/z range that contains the active site Cys723 peptide (residues 710–729) is depicted. Reversely oxidized Cys723 peptide was detected in reduced form (m/z 2075.98, *), the sulfonic acid containing peptide shifted +32.00 to m/z 2107.98 (**), and the sulfonic acid containing peptide +48.00 to m/z 2123.98 (***) reduced Cys723 containing peptide was detected in alkylated form with a shift in relative molecular mass of +57.02 (m/z 2133.00, ** **). Peak intensities of reduced and reversely oxidized Cys723 peptides were determined from the MALDI-ToF spectra (panels on the right). Relative oxidation (y axes run from 0 to 100%) is plotted against increasing H2O2 concentrations (0, 31, 62, 125, 250, 500, and 1000 μM, left to right) for wild type (WT) and mutant RPTPα-D2 (as indicated).

Fig. 4. Differential oxidation of other PTPs. PTP1B, LAR-D1, LAR-D2, RPTPα-D1-Q474G, RPTPα-D2-H764G, and PTPμ-D1 were treated with increasing concentrations of H2O2 (31–1000 μM), and reversible oxidation was detected as in Fig. 1. nc, negative control; pc, positive control.

The oxidation and PTP activity results (Figs. 2 and 3) demonstrate that the two residues that determine the difference in catalytic activity between RPTPα-D1 and RPTPα-D2 are also responsible for the difference in oxidizability, which may suggest that PTP catalytic activity and oxidizability are linked.

Oxidizability of PTP1B and LAR—Our results suggest an inverse relation between catalytic activity and oxidizability. However, other PTPs that are catalytically active have been reported to be oxidized. Therefore, we investigated oxidation of PTP1B in parallel to RPTPα-D1 and RPTPα-D2. Oxidation of zebrafish PTP1B in which the oxPTP epitope (VHCSAG) is fully conserved (31) was readily detected at very low levels of H2O2 (31 μM), and PTP1B oxidation was maximal at 125–250 μM H2O2 (Fig. 4), similar to RPTPα-D2 (Fig. 1C). PTP1B is catalytically active (data not shown), arguing against an inverse correlation between catalytic activity and oxidation.

Next, we investigated oxidizability of the two catalytic domains of the RPTP, LAR. Both LAR-D1 and LAR-D2 were readily oxidized at low H2O2 concentrations (31 μM) with maximal oxidation at 125–250 μM H2O2 (Fig. 4), similar to RPTPα-D2 (Fig. 1C). PTP1B is catalytically active (data not shown), arguing against an inverse correlation between catalytic activity and oxidation.

Because the Tyr(P) loop and WD loop are conserved in PTP1B and LAR-D1, other features of the PTPs must play a role in determining the sensitivity to oxidation. Gly259 in PTP1B (Gln747 in RPTPα-D1 and His764 in RPTPα-D2) is an important determinant of substrate specificity (34) and determines the flexibility of Gln762 in the Q loop of the catalytic pocket (35). Gly259 might therefore be involved in the difference in oxidizability between PTP1B and RPTPα-D1. We mutated Gly747 in RPTPα-D1 and His764 in RPTPα-D2 to glycine, the residue at the corresponding position in PTP1B. RPTPα-D1-Q474G was poorly oxidized (Fig. 4), like wild type RPTPα-D1 (Fig. 1B). RPTPα-D2-H764G was readily oxidized (Fig. 4), like wild type RPTPα-D2 (Fig. 1C). Taken together, these results indicate that Gly259, a determinant of substrate specificity in PTP1B and RPTPα, is not involved in differential oxidation of PTPs.

Extensive comparison of the primary sequences of the PTPs involved did not lead to the identification of residues that might be responsible for the difference in oxidizability, except for three residues that are conserved in RPTPα-D1 (Glu555, Thr690, and Asp694) but not in RPTPα-D2 (Glu678, Thr690, and Asp694) but not in RPTPα-D2 (Glu678, Thr690, and Asp694). Taken together, these results indicate that Gly259, a determinant of substrate specificity in PTP1B and PTPα, is not involved in differential oxidation of PTPs.

pH-dependent PTP Oxidation—Cysteine residues are most vulnerable to oxidation in the thiolate anion form. Therefore, the pH may be an important determinant for oxidation of PTPs.

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**Fig. 4. Differential oxidation of other PTPs.** PTP1B, LAR-D1, LAR-D2, RPTPα-D1-Q474G, RPTPα-D2-H764G, and PTPμ-D1 were treated with increasing concentrations of H2O2 (31–1000 μM), and reversible oxidation was detected as in Fig. 1. nc, negative control; pc, positive control.
It is well known that the catalytic activity of PTPs is strongly dependent on pH (36, 37). We assayed the PTP activity of RPTPα-D1, RPTPα-D2, RPTPα-D2-V555Y/E690D, and PTP1B at different pH. PTPs are highly active at low pH (optimum activity at pH 5.5–6.0 for all PTPs tested). PTP activity decreased rapidly with increasing pH to 10% of the maximum activity at pH 8.0. It is noteworthy that although the absolute oxidizability of PTPs is pH-dependent, the relative PTP activity is different (PTP1B activity at pH 8.0). It is noteworthy that although the absolute oxidizability of PTPs is pH-dependent, the relative PTP activity is different (PTP1B activity at pH 8.0). It is noteworthy that although the absolute oxidizability of PTPs is pH-dependent, the relative PTP activity is different (PTP1B activity at pH 8.0).

To investigate whether oxidation is a pH-dependent process, H₂O₂ treatment was done at a range of different pHs. Three H₂O₂ concentrations were used that are indicative of oxidizability: 31, 62, and 125 μM at pH 6.5–9.0, and reversible oxidation was assessed as in Fig. 1. nc, negative control; pc, positive control.

**DISCUSSION**

Oxidation is an attractive regulatory mechanism for PTPs, because ROS are readily formed in response to various stimuli, and oxidation is reversible. Here, we report that PTPs were oxidized differentially, which may be important for fine-tuning of the cellular responses to stimuli. We demonstrate that oxidizability and catalytic activity of RPTPα-D1 and RPTPα-D2 were reversed upon mutation of two residues in the Tyr(P) loop and WPD loop that are absolutely conserved in catalytically active PTPs but not in RPTP-D2s. However, these two residues cannot explain the difference in oxidizability between RPTPα-D1 on the one hand and the catalytically active PTPs, PTP1B, and LAR-D1 on the other, because these residues are conserved in all active PTPs. We found that oxidation of PTPs was highly dependent on pH, and we identified the PTP loop arginine as an important determinant in oxidizability.

**PTP loop Arginine Is Important for Oxidizability**—The crystal structures of the PTPs involved have all been solved: RPTPα-D1 (38), RPTPα-D2 (11), PTP1B (9), and LAR-D1 and LAR-D2 (10). Comparison of the microenvironment of the catalytic cysteine of these five PTPs indicated that the absolutely conserved PTP loop arginine is oriented differently in RPTPα-D1 than in RPTPα-D2, PTP1B, or LAR-D1 and LAR-D2 (10). We determined the distance between the Sγ atom of the catalytic cysteine and the three N atoms of the guanidinium group of the arginine (Table I). Strikingly, the Nη1 atom was much closer to the Sγ atom of the catalytic cysteine in RPTPα-D1 than in the other PTPs (4.9 Å versus 7.1–8.0 Å; Table I). The Nε1 atoms were located at similar distances in all five PTPs (5.2–6.1 Å), whereas the Nη2 atoms were partially buried in most structures at similar distances to the Sγ atom (6.1–7.3 Å). The proximity of the Nη1 atom to the Sγ atom may account for the difference in oxidizability between RPTPα-D1 and other PTPs, because the guanidinium group of Arg⁷²⁸ may sterically hinder the reaction of H₂O₂ with the thiolate group of Cys⁴³⁵.

We analyzed the crystal structures of other classical PTPs, RPTPμ-D1, TC-PTP, Shp-1, Shp-2, PTP-SL, and YOP51. We found that only in RPTPμ-D1, was the guanidinium group of the PTP loop arginine oriented similarly as in RPTPα-D1 (Fig. 6), and the Nη1 atom was relatively close to the Sγ atom (5.5 Å; Table I). Indeed, RPTPμ-D1 was only oxidized at high H₂O₂ concentrations (Fig. 4), similar to RPTPα-D1. Our results are consistent with a crucial role for the PTP loop arginine in the oxidizability of PTPs.

**Fig. 5. PTP oxidizability is pH-dependent.** A, PTP activity was assayed at pH 5.5–8.0 and is depicted relative to the maximal activity. ×, RPTPα-D1; ▲, RPTPα-D2; ●, RPTPα-D2-V555Y/E690D; ●, PTP1B. B, PTPs were treated with increasing concentrations of H₂O₂ (31, 62, or 125 μM) at pH 6.5–9.0, and reversible oxidation was assessed as in Fig. 1. nc, negative control; pc, positive control.
change in RPTPα-D2 is initiated by cyclic sulfenamide formation, which leads to extensive reorganization of the catalytic site of PTP1B (19, 20).

Although oxidizability of RPTPα-D1 and RPTPα-D2 was reversed upon mutation of only two residues in the Tyr(P) loop and WPD loop (Figs. 2 and 3), this cannot explain the difference in oxidizability between PTP1B, LAR-D1, and RPTPα-D1 (Fig. 4), because the Tyr(P) loop and WPD loop are conserved in all three active PTPs. Oxidation of PTPs is strongly dependent on pH (Fig. 5), suggesting that the microenvironment of the catalytic cysteine is an important determinant for oxidizability. Comparison of the crystal structures of the classical PTPs suggested that the orientation of the PTP loop arginine was important. The orientation of the PTP loop arginine in RPTPα-D1 suggested that RPTPα-D1 would not be highly susceptible to oxidation. Indeed, like RPTPα-D1, RPTPμ-D1 was relatively insensitive to oxidation (Fig. 4). The proximity of the PTP loop arginine to the catalytic cysteine may sterically hinder the reaction of H$_2$O$_2$ with the thiolate anion group. To directly test the role of the PTP loop arginine in oxidizability, we mutated Arg$^{439}$ to lysine in RPTPα-D1. Unfortunately, GST-RPTPα-D1-R439K was not stable, and we did not obtain sufficient material to determine the oxidation of this fusion protein.

It is noteworthy that differential oxidation of PTPs is especially obvious at relatively low H$_2$O$_2$ concentrations. Cellular

**Table I**

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**Fig. 6.** Structural basis for differential oxidation of PTPs. Shown is a comparison of the microenvironment of the catalytic cysteine in RPTPα-D1, RPTPα-D2, LAR-D1, LAR-D2, PTP1B, and RPTPμ-D1. The PTP loop is in red, the Tyr(P) loop is in green, the Q loop is in blue, and the WPD loop is in olive green. Note the difference in orientation of the guanidinium group of Arg$^{439}$ in RPTPα-D1 and Arg$^{1101}$ in RPTPμ-D1, compared with the PTP loop arginine in the other PTPs.
studies induce H$_2$O$_2$ concentrations in a range, similar to the H$_2$O$_2$ concentrations we have used for our experiments. For instance, platelet-derived growth factor induces ROS levels in cells that are comparable with the levels in cells resulting from exogenously added H$_2$O$_2$ concentrations of 0.1–1.0 mM (21). Differential oxidation of PTPs is important for fine-tuning of the cellular response to ROS production. Apparently, different inactivating mechanisms have evolved in the RPTPs: direct oxidation of the catalytic cysteine in LAR and indirect inactivation through oxidation of the catalytic cysteine in RPTPα-D2, resulting in stabilization of an inactive dimer conformation (8, 29). LAR-D2 does undergo a conformational change in response to H$_2$O$_2$, like RPTPα-D2, and H$_2$O$_2$ induces heterodimer formation between full-length RPTPs and LAR (44). Whether oxidation of LAR-D2 is required for full inactivation of LAR remains to be determined.

In conclusion, we demonstrate that PTPs are differentially oxidized, and we provide evidence for the underlying mechanism. It will be interesting to see whether other PTP family members, including the nonclassical Cys-based PTPs, are differentially oxidized as well.

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