Minireview

Redox regulation of protein-tyrosine phosphatases

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Abstract

The protein-tyrosine phosphatases (PTPs) form a large family of signaling proteins with essential functions in embryonic development and adult physiology. The PTPs are characterized by an absolutely conserved catalytic site cysteine with a low pK_a due to its microenvironment, making it vulnerable to oxidation. PTPs are differentially oxidized and inactivated in vitro and in living cells. Many cellular stimuli induce a shift in the cellular redox state towards oxidation and evidence is accumulating that at least part of the cellular responses to these stimuli are due to specific, transient inactivation of PTPs, indicating that PTPs are important sensors of the cellular redox state.

Phosphorylation of proteins on tyrosine residues is one of the most important regulatory cell signaling mechanisms, controlling cell proliferation, differentiation, and migration. Cellular phosphotyrosine levels are regulated by the antagonistic activities of two classes of enzymes, the protein-tyrosine kinases (PTKs)[1] and the protein-tyrosine phosphatases (PTPs). Until recently, little was known about regulation of PTPs, but now oxidation is emerging as an important regulator of PTPs.

The catalytic cysteine of PTPs is a target for oxidation

The human genome encodes approximately 100 genes that belong to the PTP superfamily with conserved catalytic sequences, including the absolutely conserved active site cysteine. Approximately 40 of these genes encode the classical PTPs that are highly specific for phosphotyrosine [1]. The other PTP superfamily members include the low molecular weight (LMW) PTPs, dual specificity phosphatases (DSPs), and lipid phosphatases, among which is the important tumor suppressor PTEN [2]. Roughly half of the classical PTPs contain a single transmembrane domain and are tentatively called receptor PTPs (RPTPs). Most RPTPs have two conserved cytoplasmic PTP domains of which the one closest to the cell membrane (D1) contains most—if not all—catalytic activity [3]. The membrane distal PTP domain (D2) has a regulatory role.

Due to their microenvironment, the catalytic cysteines have a low pK_a [4,5]. Under normal conditions, the active site cysteines are in the thiolate anion form and therefore, they are highly susceptible to oxidation. By now, members of each subfamily have been shown to be oxidized by treatment with oxidizing agents (e.g., H_2O_2), including classical PTPs [6], LMW PTPs [7], DSPs [8], and lipid phosphatases [9]. Oxidation of the catalytic cysteine blocks the capacity of these enzymes to dephosphorylate their targets, because catalysis is mediated by transfer of the phosphate moiety from the substrate to the catalytic cysteine, followed by rapid hydrolysis of the phosphate [10]. In general, oxidation of
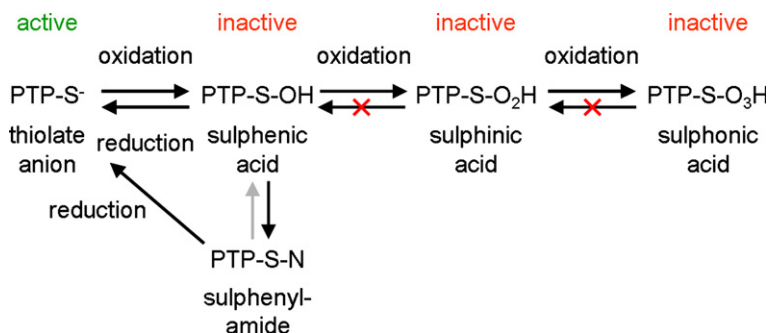

Fig. 1. Oxidation of PTPs. The catalytic cysteine is in the thiolate anion form under normal conditions due to its low pK_a. Oxidation leads to sulfenic acid formation and further oxidation leads to irreversible sulfinic and sulfonic acid formation. Sulfenic acid is rapidly converted to cyclic sulfenylamide. Sulfenylamide cannot be further oxidized to sulfinic or sulfonic acid, but can be reduced to the thiolate anion form in response to thiols. Only the reduced form is catalytically active.

cysteine residues to sulfenic acid is reversible, while highly oxidizing conditions will induce further oxidation to sulfinic and sulfonic acid, which is irreversible [8]. Recently, a novel bond was found to be formed upon oxidation of the classical cytoplasmic PTP1B, which was termed sulfenylamide or sulfenamide [11,12]. The crystal structure of oxidized PTP1B revealed that sulfenamide is characterized by a five-membered ring that forms by binding of the sulfur of the cysteine to the backbone nitrogen of the neighboring serine residue (Fig. 1). Conversion of sulfenic acid to sulfenamide is fast, because sulfenic acid was not observed in PTP1B crystals upon oxidation. Interestingly, sulfenamide—unlike sulfenic acid—is not easily further oxidized to sulfinic and sulfonic acid. Given the high conservation of the catalytic sites in the PTP family, cyclic sulfenamide formation may be a general mechanism of oxidation. Cyclic sulfenamide can be reduced by thiols, including glutathione. The sulfenic acid form and presumably also the cyclic sulfenamide form of PTP1B is readily converted in the presence of glutathione to the more stable S-glutathionylated PTP1B [13], which can be reduced by DTT or thioltransferase.

Cyclic sulfenamide formation protects classical PTPs against inadvertent irreversible further oxidation to sulfinic and sulfonic acid. S-glutathionylation also prevents irreversible oxidation of PTPs. Another protective mechanism that was discovered in non-classical PTP superfamily members is disulfide bond formation. In LMW-PTP, CDC25, and PTEN, intramolecular disulfide bonds involving the catalytic cysteine and an adjacent cysteine have been observed [7,9,14]. Intramolecular disulfide bond formation involving the catalytic cysteine of classical PTPs has not been reported to date.

Physiological stimuli that regulate PTPs by oxidation

Many stimuli, including growth factors, cytokines, and ultraviolet (UV) light, induce the production of reactive oxygen species (ROS). Evidence is accumulating that ROS production in response to growth factors is mediated by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase protein complex (for reviews, see [15,16]). Intracellular ROS levels in response to natural stimuli have been determined using fluorescent ROS indicators, such as derivatives of dichlorofluorescein (DCF). For instance, platelet-derived growth factor (PDGF) induces ROS levels in cells that are comparable to the levels in cells resulting from exogenously added H_2O_2 concentrations of 0.1–1.0 mM [17]. Given the sensitivity of PTP catalytic cysteines to oxidation in vitro, these PDGF-induced ROS levels are more than sufficient to oxidize active site cysteines in PTPs.

Growth factor signaling is actually dependent on ROS production, since ROS quenching abolishes growth factor signaling [17,18]. Epidermal growth factor (EGF)-induced ROS production coincides with inhibition of PTP1B activity [18]. Inactivation of PTPs by growth factor-induced oxidation would explain why ROS are essential for growth factor signaling. In fact, mathematical analysis of the minimal reaction network involving a growth factor receptor PTK on the one hand and PTPs that are inactivated by oxidation upon PTK activation on the other mimics experimental EGFR phosphorylation in cells [19], corroborating the PTK-induced ROS-mediated PTP inactivation model. Meng et al. [20] provided compelling evidence for the growth factor-induced PTP inactivation model using a modified in gel phosphatase assay that allows detection and identification of oxidized PTPs. This assay was validated using H_2O_2 that induces oxidation of multiple PTPs in Rat-1 cells in a concentration-dependent manner. The SH2-containing PTP, Shp-2, is specifically and transiently oxidized in response to PDGF in Rat-1 cells. PDGF-induced signaling is reduced in the presence of N-acetylcysteine, a ROS-quencher, indicating that ROS-mediated inhibition of PTPs is essential for PDGF signaling [20]. Association of Shp-2 with the PDGF

receptor is required for Shp-2 oxidation, indicating that this is a localized effect. Strict subcellular localization of PTP oxidation provides specificity to the PDGF response in that not all PTPs throughout the stimulated cell are inactivated, but only the ones very close to the PDGF receptor.

Using an antibody that allows detection of oxidized active site cysteines in PTPs, anti-oxPTP, we demonstrated that PDGF treatment indeed induced oxidation of Shp-2 [21]. This antibody was raised against the sulfonic acid form of a hexapeptide containing the catalytic site cysteine (VHC*SAG) that is highly conserved in classical PTPs. Oxidized active site cysteines are detected by (1) alkylation to irreversibly block the reduced cysteines, (2) reduction to allow step, (3) triple oxidation to the sulfonic acid form, and (4) detection using the anti-oxPTP antibody. Using this antibody, we provided direct evidence for growth factor signaling-induced oxidation of a PTP.

Oxidation-induced PTP inactivation may not only be involved in growth factor receptor activation, but also in other stimuli that induce ROS production. It is noteworthy that UV, ionizing irradiation, and G protein coupled receptor activation stimulate ROS production and induce receptor PTK transactivation [22–24] that may be explained by PTP inactivation. PTP activity assays indicated that UV treatment leads to partial inactivation of PTPs, including Shp-1 and the transmembrane RPTPα, Dep-1, and RPTPσ. UV-induced inactivation of these PTPs is blocked by *N*-acetylcysteine, indicating that ROS are required for the effect [25]. Using the anti-oxPTP antibody, we demonstrated that UV treatment of cells leads to oxidation of RPTPα [21], providing direct evidence for UV-induced oxidation of a PTP.

Given the high sensitivity of PTPs to changes in the cellular redox state, it is conceivable that PTPs act as cellular redox sensors, mediating the initial response to changes in redox state following stimulus-induced ROS production. This notion is consistent with stimulus-induced enhanced tyrosine phosphorylation in cells, which may be the direct result of PTP inactivation. Definitive proof that ROS-induced inactivation of PTPs is essential for stimulus-induced signaling will require development of mutant PTPs that are not inactivated by oxidation. If such mutants block signaling, it is evident that inactivation of PTPs is required for stimulus-induced signaling.

Unexpected regulation of RPTPs by oxidation

RPTPs are negatively regulated by oxidative stress. We found that the active site cysteine in RPTPα-D1 is not directly oxidized and that oxidation-induced inhibition of RPTPα is regulated in an unexpected manner.

RPTPs—like many other single membrane-spanning receptors—are regulated by dimerization. Ligand-induced dimerization of a fusion protein between the epidermal growth factor receptor and the RPTP, CD45, leads to functional inactivation [26]. Moreover, enforced dimerization of RPTPα by introduction of a disulfide bond in the extracellular domain leads to inactivation of RPTPα, depending on the exact position of the disulfide bond [27]. The relative orientation of the two monomers in the dimer, rotational coupling, apparently has a role in dimerization-mediated inactivation. The crystal structure of RPTPα-D1 indicated that a helix–loop–helix wedge-like structure to the N-terminal side of D1 occluded the catalytic site of the other monomer and vice versa, providing structural evidence for dimerization-mediated inactivation [28]. Mutations in the wedge-like structure abolished dimerization-induced inactivation of both CD45 and RPTPα [27,29]. Therefore, RPTPs can be regulated by dimerization. We and others have demonstrated that RPTPs dimerize extensively in living cells, using cross-linkers and fluorescence resonance energy transfer (FRET) between fusion proteins of RPTPα and derivatives of green fluorescent protein (GFP) [30–33]. Moreover, we found that multiple domains in RPTPs, including the individual PTP domains, the transmembrane domain, and the extracellular domain, are involved in dimerization.

Recently, we found that RPTPα dimerization is regulated by oxidation (Fig. 2) in that oxidative stress induces stabilization of RPTPα dimers. RPTPα-D2 undergoes a conformational change in response to oxidative stress, which is dependent on the active site cysteine in D2 (Cys723) [34]. The conformational change in RPTPα-D2 induces a change in rotational coupling, observed in the extracellular domain [35], resulting in stabilization of RPTPα dimers and inactivation of RPTPα. Surprisingly, RPTPα-C723S is only partially inactivated in response to oxidative stress [34]. Cys723 is essential for the conformational change in RPTPα-D2, for stabilization of dimerization as well as the change in rotational coupling, suggesting that these effects are responsible for complete inactivation of wild type RPTPα. Using the anti-oxPTP antibody, we demonstrate that Cys723 in RPTPα-D2 is much more sensitive to oxidation in vitro than Cys433 in RPTPα-D1 [21]. In cells, Cys723, but not Cys433, is oxidized in response to UV [21]. This implies that PTPs in general may be differentially oxidized. Based on these results, we propose a model for regulation of RPTPα (Fig. 2). Briefly, oxidizing conditions induce oxidation of Cys723 in RPTPα-D2, leading to a conformational change which triggers rotation of the two monomers in the dimer relative to each other, thus stabilizing the dimer in an inactive conformation. Oxidation-induced inactivation is reversible. Mutation of Cys723 abolishes all of these effects, including stabilization of the dimers and inactivation of RPTPα catalytic activity. Taken together, RPTPα is inactivated by oxidation, even though the catalytic Cys433 is not oxidized
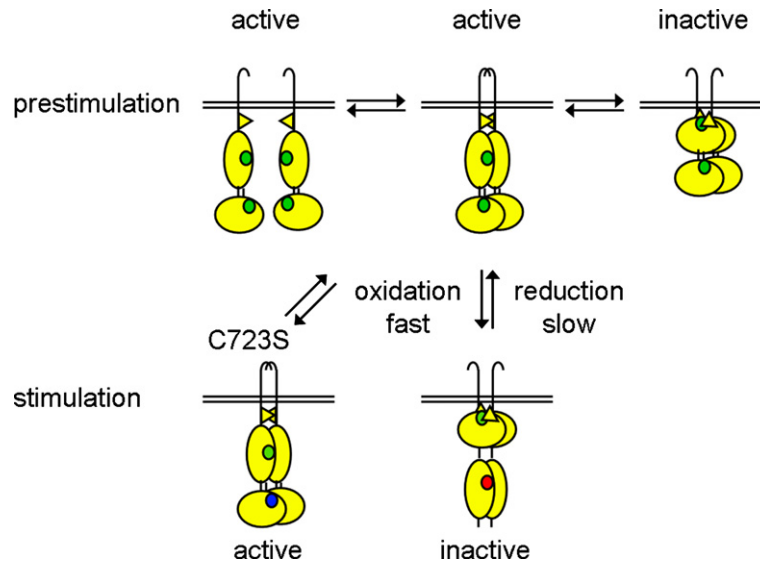
Fig. 2. Inactivation of RPTPα by oxidation. Under normal conditions (prestimulation), active RPTPα monomers are in equilibrium with active and inactive dimers. Oxidation rapidly (within 5 min) induces oxidation of the active site cysteine in D2 (Cys723, red), not D1. With similar kinetics dimers are stabilized and rotational coupling is altered, resulting in inactivation. After removal of the source of oxidative stress, all of these parameters slowly (~2 h) return to prestimulation levels. The rate-limiting step here is not clear. It is likely that reduction of Cys723 is rapid and that the conformational change of D2 and dimer destabilization is slow. Mutation of the active site cysteine in D2 (C723S, blue) abolishes all of the effects, observed in wild type RPTPα and RPTPα-C723S is only partially inactivated by oxidative stress.

directly. It will be interesting to see if other RPTPs are regulated by oxidation in a similar fashion.

Conclusions

Oxidation is emerging as an important regulatory mechanism for the PTP family of enzymes. Many stimuli induce ROS production, shifting the redox state of cells. PTPs are highly sensitive to changes in the cellular redox state and it is conceivable that the cellular responses to stimulus-induced ROS production are the result of PTP inactivation. The specificity of the response may result from differential PTP inactivation due to intrinsic differences in sensitivity to oxidation, or due to differences in (sub)cellular localization of the source of ROS on the one hand and of the PTPs on the other.

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