Regulation of PTP1B via Glutathionylation of the Active Site Cysteine 215

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ABSTRACT: The reversible regulation of protein tyrosine phosphatase is an important mechanism in processing signal transduction and regulating cell cycle. Recent reports have shown that the active site cysteine residue, Cys215, can be reversibly oxidized to a cysteine sulfenic derivative (Denu and Tanner, 1998; Lee et al., 1998). We propose an additional modification that has implications for the *in vivo* regulation of protein tyrosine phosphatase 1B (PTP1B, EC 3.1.3.48): the glutathionylation of Cys215 to a mixed protein disulfide. Treatment of PTP1B with diamide and reduced glutathione or with only glutathione disulfide (GSSG) results in a modification detected by mass spectrometry in which the cysteine residues are oxidized to mixed disulfides with glutathione. The activity is recovered by the addition of dithiothreitol, presumably by reducing the cysteine disulfides. In addition, inactivated PTP1B is reactivated enzymatically by the glutathione-specific dethiolase enzyme thioltransferase (glutaredoxin), indicating that the inactivated form of the phosphatase is a glutathionyl mixed disulfide. The cysteine sulfenic derivative can easily oxidize to its irreversible sulfinic and sulfonic forms and hinder the regulatory efficiency if it is not converted to a more stable and reversible end product such as a glutathionyl derivative. Glutathionylation of the cysteine sulfenic derivative will prevent the enzyme from further oxidation to its irreversible forms, and constitutes an efficient regulatory mechanism.

Protein tyrosine phosphatases (PTPs[1]) are important enzymes in the control of cell cycles and signal transduction by acting in conjunction with protein tyrosine kinases to regulate levels of protein tyrosine phosphorylation in response to cellular signals (*1*, *2*). Protein kinases have been extensively reported in the literature, whereas protein phosphatases are only now being examined with increasing intensity (*3*, *4*). In particular, protein tyrosine phosphatase 1B (PTP1B, EC 3.1.3.48), a ubiquitous mammalian phosphatase, has been the focus of a number of studies that have elucidated both the structure and function of this protein (*5*−*8*). Recent reports have focused on the regulation of PTP1B via oxidation of the active site cysteine residue, Cys 215 (*9*, *10*). Its relatively low pK_a of ∼5.6 (*11*) makes it an ideal candidate for site-specific modifications due to the reactive nature of the thiolate anions which can mediate the phosphatase activity.

Denu and Tanner (*9*) have shown that hydrogen peroxide abolishes activity of PTP1 by forming a cysteine sulfenic derivative. The activity could then be recovered by the addition of dithiothreitol (DTT); presumably this reduced the sulfenic acid (*12*, *13*). In a separate study, the stimulation of A431 cells by epidermal growth factor (EGF) results in reversible inactivation of PTP1B via a cysteine sulfenic derivative (*10*). The oxidation of the active site cysteines of various PTPs by exogenous agonist or exogenous hydrogen peroxide has been suggested to be a potential regulatory mechanism that can account for the increased levels of protein tyrosine phosphorylation (*9*, *10*, *14*, *15*).

An important criterion in regulating phosphatase activity is that the modification be specific and reversible. The cysteine sulfenic acid may indeed undergo such reactions, although it can be oxidized further to the other nonreducible derivatives, for example, cysteine sulfinic and cysteine sulfonic acids, within the cell. A possible reaction to prevent the further oxidation of the reversible cysteine sulfenic to its irreversible derivatives is to have the cysteinesulfenic acid or the cysteine itself react with glutathione (GSH) within the cell. A mixed disulfide formation with GSH has been shown in several proteins including the HIV-1 protease (*16*, *17*) and carbonic anhydrase (*18*). The formation of a mixed disulfide between PTP1B and GSH (or GSSG) would prevent irreversible oxidation of the active site cysteine and provide for the reversible reduction either chemically or enzymatically. We report that PTP1B is inactivated by the formation of a mixed disulfide with glutathione and that this inactivation is reversed not only by DTT but also more importantly by thioltransferase, a thiol-disulfide oxidoreductase that is specific for glutathionyl mixed disulfide substrates (*19*, *20*)

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[1] Abbreviations: PTP, protein tyrosine phosphatase; PTP1B, protein tyrsoine phosphatase 1B; DTT, dithiothreitol; EGF, epidermal growth factor; GSH, reduced glutatathione; GSSG, glutathione disulfide; Da, Daltons; DTPA, diethylenetriaminepentacetic acid; PDI, protein disulfide isomerase; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); PFK, phosphofructokinase.

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and specifically utilizes GSH as cosubstrate (*21*). The glutathionylation occurs on Cys215, the active site cysteine. This mechanism suggests an alternative modification to the redox regulation of cysteine in PTP1B and suggests a possible *in vivo* mechanism in the regulation of phosphatase activity.

EXPERIMENTAL PROCEDURES

Materials. Chelex 100 Resin (200−400 mesh, sodium form) was from Bio-Rad Laboratories. Glutathione, DTT, diamide, DTNB, DHB, and DTPA were from Sigma Chemical Co. The synthetic peptide (DADEpYLIPQQG, corresponding to $EGFR_{988-998}$) was from Peptide Technologies Corporation (Gaithersburg, MD). Sequencing grade trypsin was from Boehringer-Mannheim. Recombinant PTP1B (composed of amino acids 1−321) was prepared as described previously (*22*). Dr. John J. Mieyal of Case Western University kindly provided purified thioltransferase.

Assay of PTP1B Activity. The activity of PTP1B was monitored using a spectrofluorometric assay previously described (*23*). The peptide substrate was incubated at 30 °C in 50 mM Tris, 150 mM NaCl, 0.1 mM DTPA, pH 7.0, buffer that was chelexed overnight. An aliquot of PTP1B was added to substrate, and activity was monitored by the increase in fluorescence at 305 nm with an excitation wavelength of 280 nm. Inactivation of PTP1B by GSSG was carried out by incubating 500 nM protein in the presence of 25 mM glutathione disulfide. When inactivated by diamide and reduced glutathione, 500 nM PTP1B was incubated in the presence of 1 mM diamide and 2 mM GSH. Aliquots of PTP1B were removed at various time points and added to the substrate, adjusted to a final enzyme concentration of 5 nM, and assayed for activity. The total reaction volume was 100 μL. Kinetic constants are calculated as a pseudo-first-order process using MLAB (Civilized Software Inc., Bethesda, MD), where k is the rate constant of inactivation, and [GSSG] is in large excess relative to [PTP].

$$\frac{d[PTP]_{inactive}}{dt} = k[PTP][GSSG]$$

The GSSG-inactivated sample of PTP1B was exchanged three times with buffer in Microcon-30 (Amicon) to remove excess GSSG. The sample was then incubated with either 10 mM DTT or 120 milliunits of thioltransferase which corresponds to 0.1 nmol (1 μM in 100 μL) and 0.5 mM GSH, and at various time points an aliquot was removed and assayed for activity. The diamide/GSH-inactivated sample was exchanged three times using a Microcon-30 (Amicon) to remove excess diamide and GSH. Similar to the GSSG sample, 10 mM DTT was then added to the sample and analyzed for activity at various time points.

Analysis of PTP1B by Gel Electrophoresis. Samples of PTP1B were inactivated by either diamide/GSH treatment or glutathione disulfide. A separate set of samples was inactivated by similar treatment and exchanged in buffer three times with an Amicon-30 Centricon. DTT was then added to the sample and incubated for 30 min. Samples were then run on NOVEX NuPage gels according to the manufacturer's protocol.

Titration of Unmodified Cysteine Residues by DTNB. To examine the number of cysteines modified by diamide and GSSG, we inactivated the PTP1B samples by diamide/GSH or GSSG for 1 h. Excess diamide/GSH or GSSG was removed by performing three buffer exchanges in an Amicon-30 Centricon. The modified PTP1B was then titrated with DTNB according to published procedures (*24*). Samples were also analyzed spectrophotometrically at 410 nm using a Hewlett-Packard 8453 spectrophotometer. The results were related to a standard curve generated using β-mercaptoethanol as the standard.

Mass Spectrometry Analysis of Modified PTP1B. Samples for ESI-MS were exchanged three times with water in an Amicon-30 Centricon to remove as much buffer and salts as possible. Acetic acid was added to the samples to give a final concentration of 10%. The on-line microdialysis technique was used as described previously (*25*). The modified PTP1B was examined using the TSQ-700 electrospray mass spectrometer (Finnigan MAT, San Jose, CA). The heated capillary of the mass spectrometer was maintained at 200 °C, and the spray voltage was 5 KV. Samples were subjected to on-line dialysis versus 1:1 v:v methanol/water containing 5% acetic acid. The syringe was driven using a Harvard Apparatus syringe pump (South Natick, MA). The spectra were acquired for 1 min, and the data were deconvoluted using Finnigan software.

The procedure used for *in-gel* trypsin digestions and peptide extractions has been described previously (*26*). This procedure was modified as follows: the excised gel band was soaked for at least 12 h in a solution of 50:40:10, methanol/water/acetic acid (v:v:v) prior to digestion. Sequencing grade trypsin was used for digestions in a water bath at 37 °C for 2 h. Consecutive extractions with 30 μL of acetonitrile were pooled and then dried in a Speed Vac (Savant, Holbrook, NY) evaporator before being redissolved in 50:50 acetonitrile/water. A 0.5 μL sample of this solution was then spotted on MALDI plates with equal volume of a saturated solution of 2,5-dihydroxybenzoic acid (DHB) matrix that was diluted 1:3 (v:v) with 50% acetonitrile/50%water. MALDI-TOF analysis was performed on a PerSeptive Biosystems (Framingham, MA) Voyager DE STR instrument. The MALDI spectra produced for both the digested control and oxidized samples were the average of 128 laser shots. The spectra of oxidized samples were directly compared with the spectra produced by a control sample (wildtype, no added oxidizing reagents).

Liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis was performed when additional confirmation of oxidation was required through peptide sequencing. A Michrom BioResources (Auburn, CA) Magic 2002 HPLC was coupled on-line to a Finnigan LCQ quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA) through its electrospray interface. A capillary 50 mm × 200 μm column filled with C18 (5 μm particle diameter, 200 Å pore size) was used with mobile phases of A (4:95:1, methanol/water/acetic acid) and B (85:14:1, methanol/water/acetic acid). The flow was split precolumn with a Magic capillary splitter assembly (Michrom BioResources, Auburn, CA). Fragmentation data (MS/MS) were acquired in centroid format using the data-dependent mode where a preselected set of ions are fragmented after a threshold of at least 5×10^5 counts (as detected by the LCQ software) is exceeded. During the LC/MS/MS analysis this was repeated twice for a total of three scans for averaging. As with the MALDI-

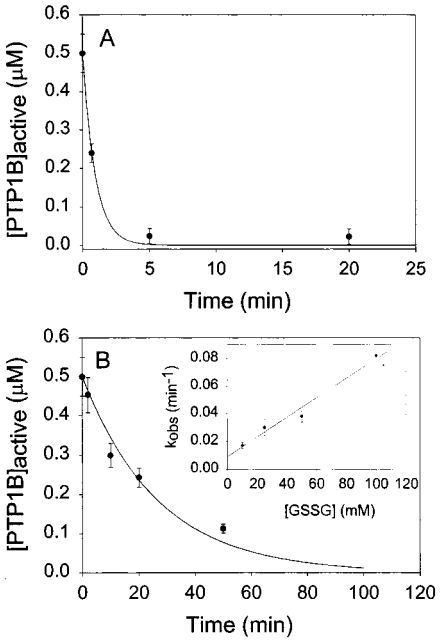

FIGURE 1: (A) Time-dependent inactivation of 0.5 μM PTP1B by 1 mM diamide and 2 mM GSH at 30 °C; the line was calculated first-order time course with a rate constant of 1.1 min^{-1}. (B) Time-dependent inactivation of 0.5 μM PTP1B by 50 mM GSSG; the line represents a calculated first-order inactivation with a pseudo-first-order rate constant of 0.038 ± 0.004 min^{-1}. (inset) k_{obs} for the inactivation by various concentrations of GSSG with a second-order rate constant of 0.012 M^{-1} s^{-1}.

TOF data, the peptide fragment ions that contain the oxidized cysteine are increased in mass of 305 Da by the addition of glutathione. The mass isolation window for parent ions was 4 Da, the scan range (during parent ion scan) was *m*/*z* 200−2000, and the collision energy was set to 32%.

RESULTS

Reversible Inactivation of PTP1B by Mixed Disulfide Formation with Glutathione. PTP1B was tested for inactivation by modification of cysteine residues with glutathione. Diamide, a strong RSH oxidant (*27*), was used in the presence of 2 mM GSH. The inactivation occurred within 5 min, and the enzyme remained inactive over time (Figure 1A). Diamide alone had no effect on phosphatase activity (data not shown). The inactivation followed first-order kinetics with a pseudo-first-order rate constant of 1.1 ± 0.1 min^{-1}. The second-order rate constant was calculated to be 18.3 M^{-1} min^{-1} for the inactivation reaction, suggesting a rapid formation of mixed disulfide. Since it is proposed that PTP1B is regulated by a redox mechanism, we examined whether the disulfide modification was reversible by adding 10 mM DTT; within 30 min, more than 90% of the phosphatase activity was restored (data not shown).

The rate of inactivation with GSSG is much slower than that of the chemically induced disulfide formation by diamide. The inactivation followed first-order kinetics with a pseudo-first-order rate constant of 0.038 ± 0.004 min^{-1} when 50 mM GSSG was used (Figure 1B). From this rate constant and the concentration of GSSG used, the second-order rate constant was calculated to be 0.76 M^{-1} min^{-1} for the inactivation reaction, indicating a slow formation of mixed disulfide. This rate can be significantly accelerated by a reactive species such as diamide as shown in Figure 1A. The value of the pseudo-first-order rate constant for the inactivation of PTP1B is linearly proportional to the concentration of glutathione when varied from 10 to 100 mM (Figure 1B inset). The slope yields a second-order rate constant of 0.72 M^{-1} min^{-1} for the forward reaction.

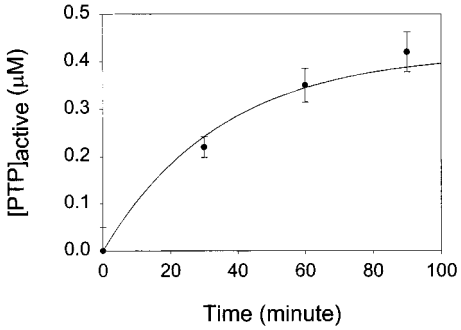

FIGURE 2: Time-dependent reactivation of PTP1B by thioltransferase. The reaction mixture contained 50 mM Tris, 150 mM NaCl, 0.1 mM DTPA, 0.5 mM GSH, pH 7.0, and 0.5 μM GSSG-inactivated PTP1B. The reaction was initiated by the addition of 1 μM thioltransferase. The line represents the calculated first-order reactivation reaction with a rate constant of 0.029 min^{-1}.

Addition of 10 mM DTT to the GSSG-inactivated enzyme caused the enzyme to restore more than 80% of the original phosphatase activity (data not shown). Furthermore, in a separate study the enzyme thioltransferase was shown to restore phosphatase activity of a GSSG-inactivated enzyme. Approximately 80% of the original phosphatase activity was recovered by the enzyme-catalyzed disulfide exchange with thioltransferase. The reactivation of PTP1B by thioltransferase obeyed first-order kinetics, with a rate constant of 0.029 ± 0.003 min^{-1} (Figure 2). The maximal activity restored occurred at about 90 min. The slow recovery of the activity may be a result of suboptimal conditions for thioltransferase or inefficient exchange of inactivators.

DTNB Titration of PTP1B. The PTP1B samples were glutathionylated as described above. DTNB was added to the samples and incubated for 1 h. The samples were analyzed by UV/vis spectroscopy. A total of six molar equivalents of cysteine were calculated for PTP1B, which is in agreement with the sequence in which there are six cysteine residues (no disulfides). The PTP1B sample treated with GSSG had 3.6 molar equiv of titratable cysteines, indicating that 2.4 molar equiv cysteine had been blocked, while the PTP1B treated with diamide/GSH had no titratable cysteine residues indicating that all six cysteine residues were blocked, presumably by glutathionyl disulfides.

Analysis of Modified PTP by SDS−PAGE Electrophoresis. When the inactivated PTP1B samples were analyzed by SDS−PAGE, the protein treated with diamide/GSH or GSSG (Figure 3, lanes 2 and 4, respectively) showed a definitive shift in mobility, suggesting that the molecular weight of PTP1B was varied. Both diamide/GSH and GSSG-modified enzyme migrate slower than the unmodified enzyme (lane 1), with diamide-mediated modified PTP1B being the slowest. This indicates that the diamide/GSH-inactivated enzyme exhibits an extensive change in its molecular weight relative to that modified by GSSG. The addition of DTT to the inactivated samples (Figure 3, lanes 3 and 5) caused the inactivated PTP1B to migrate at a rate closer to that of the untreated PTP1B. The results obtained with DTT suggest that the enzyme was modified by forming mixed disulfide

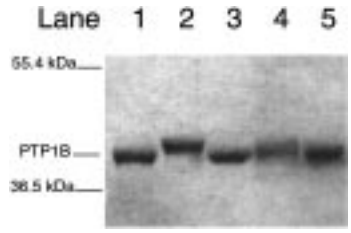

FIGURE 3: NuPAGE 4−12% gel analysis of PTP1B sample under nonreducing conditions: lane 1, control; lane 2, PTP1B treated with 1 mM diamide/2 mM GSH; lane 3, PTP1B/diamide sample treated with 10 mM DTT; lane 4, PTP1B treated with 25 mM GSSG; lane 5, PTP1B/GSSG sample treated with 10 mM DTT. The gel indicates that inactivating the sample by treatment with diamide or GSSG results in a shift in mobility of PTP1B, which is reversible by the addition of the reducing agent DTT.

bonds with glutathione, and more cysteine residues were modified when diamide was present in the reaction mixture.

Mass Spectrometry Analysis of Modified PTP1B. Subsequently the modified PTP1B proteins were analyzed by mass spectrometry to determine the nature of the modification as shown by SDS−PAGE analysis. The solution from treatment of PTP1B with diamide/GSH was analyzed by electrospray mass spectrometry (ESI/MS) and found to be 1832 Da higher in mass than the unmodified recombinant PTP1B (37316 Da), which agrees with 37312.76 as calculated from its sequence (Figure 4, panel B vs A). This result shows that PTP1B has added six glutathiones ($6 \times 305 = 1830$), indicating modification of all six cysteine residues in PTP1B. When PTP1B was treated with 25 mM GSSG, ESI/MS indicates that from one to four glutathiones form mixed disulfides with the cysteine residues of PTP1B (Figure 4C). The DTNB titration showed that a total of 2.4 molar cysteine residues is glutathionylated which is consistent with the data shown in Figure 4C. It indicates that the products contain about 40% diglutathionylated, 30% triglutathionylated, 20% monoglutathionylated, and 10% representing adducts as suggested by the shoulders on each peak. Since PTP1B is totally inactivated by both of these treatments, and the GSSG treatment contains 20% monoglutathionylated modification and the rest with higher degrees of modification, the first glutathionylated cysteine residue is the active site cysteine, Cys215, which possesses a unique low pK_a, and it is known to form a cysteine-phosphate intermediate during catalysis (*20*).

The modified PTP1B samples obtained by treating the enzyme with 25 mM GSSG were then analyzed by LC/MS/MS to determine if the active site Cys215 formed a disulfide with glutathione. The samples were digested by trypsin and analyzed on the basis of the mass change of the unmodified fragment containing Cys215 when modified by glutathione. Figure 5 (lower trace) shows the total ion current scanned from 200 to 2000 Da. The peak eluting at 3 min was fragment 28 (2479.05 Da) of the tryptic digest which contains the active site cystiene 215, and the MS/MS of its triply charged ion is shown in the upper trace. The fragments y_{13}^{+2}, y_{20}^{+2}, y_{21}^{+3}, b_{16}^{+2}, $b_{17}^{+2,*}$, b_{17}^{+2}, $b_{19}^{+3,*}$, and $b_{22}^{+2,*}$ all contain the glutathione addition to the active site cysteine, whereas the other fragments do not. The results indicate that, when PTP1B is treated with GSSG, Cys215 does indeed form a disulfide with one GSH molecule, thus inactivating the phosphatase (Figure 5).

DISCUSSION

The regulation of protein tyrosine phosphatases is an increasing area of interest as the delicate balance between PTPs and protein tyrosine kinases is vital in regulating cell cycle and signal transduction processes. The recent reports of hydrogen peroxide as a mechanism for regulation (*9*, *10*) are important to the elucidation of PTP regulation; however the highly reactive nature of peroxide is somewhat counterintuitive to redox regulation because of its known ability to damage various amino acids (*29*). While the proposed cysteine sulfenic derivatives can further oxidize to form the irreversible sulfinic and sulfonic derivatives, it can also react with glutathione to form a disulfide that is easily reversed both chemically and enzymatically as well as preventing excessive oxidation.

We have shown that PTP1B can be glutathionylated by chemical treatment with the strong oxidizing agent, diamide. The rate of glutathionylation is strongly enhanced by diamide, and the modification sites become nonselective likely due to the ability of diamide to extract a hydride from thiols to form a reactive RS^+ which can easily form a disulfide bond with another thiol such as GSH. Since oxidative signals, such as hydrogen peroxide, can result in a decrease of GSH through the formation of mixed disulfides with proteins or GSSG (*30*−*33*), the inactivation of PTP1B was examined in the presence of GSSG. The active thiol group of Cys215 in PTP1B has been shown to be able to form a mixed disulfide with GSSG in which a nucleophilic attack occurs between the disulfide of GSSG, resulting in a mixed protein disulfide. The slow formation of the glutathionylated PTP1B by GSSG at pH 7.0 supports the hypothesis that a more reactive cysteine species is needed to form the disulfide, preventing unwanted glutathionylation during normal cellular function. On the other hand, the reversible nature of the modification suggests it as a potentially important species in the regulation of protein phosphatases with active site cysteine. The formation of a stable mixed disulfide from the highly reactive cysteine sulfenic derivative provides an alternative easily reversible inactive intermediate that the cell can reduce readily with a number of enzymes including thioltransferase.

Analysis of the products of the reactions by gel electrophoresis indicated that the molecular weight of PTP1B was altered by glutathione and not by dimer formation between two subunits of PTP1B. In addition, the crystal structure of PTP1B precludes the formation of intramolecular disulfide formation (D. Barford, personal communication). Mass spectrometry confirmed that the mass of PTP1B was modified by one to six glutathiones depending upon treatment. Titration of the cysteine residues by DTNB provided a measure of the stoichiometry of cysteine residues modified; although the number does not necessarily reflect modification of a specific cysteine residue(s). However, the relative specificity of the glutathionylation by GSSG on Cys215 was shown by LC/MS/MS. This result provides another potential regulatory mechanism for phosphatase activity, since the reduced sulfhydryl of Cys215 is required for catalysis to form a cysteine-phosphate intermediate (*28*).

The glutathionylation of proteins is well-documented (*16*, *18*). With respect to protein phosphatases, it provides an attractive addition to the cysteine sulfenic acid intermediate.

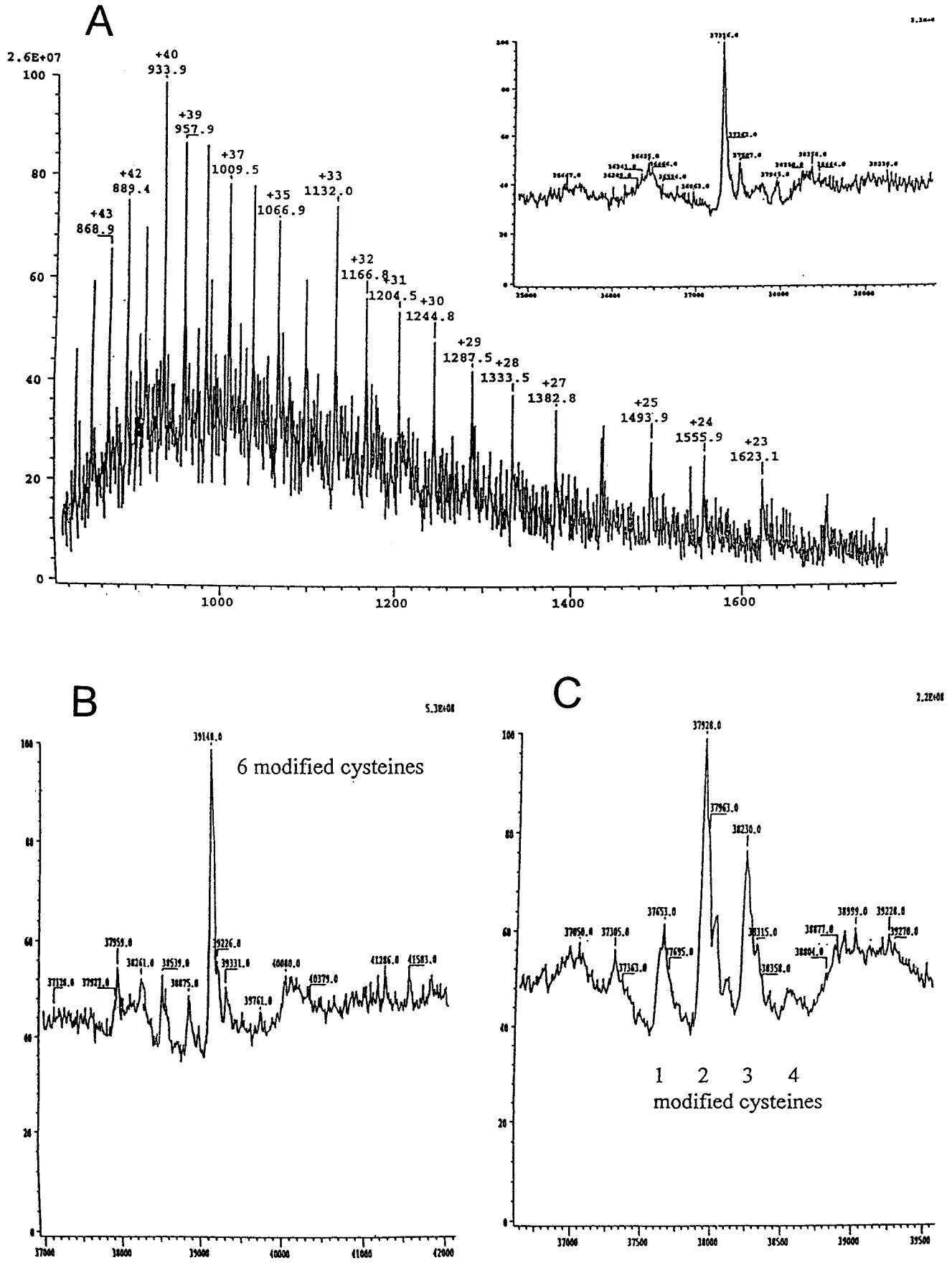

FIGURE 4: Triple quadrupole mass spectrometry ESI/MS spectra for PTP1B: (A) control PTP1B expressed as relative intensity versus *m*/*z*; (inset) the deconvoluted spectra expressed as relative intensity versus mass (note that the major peak has a mass value of 37 316); (B) diamide/GSH-treated PTP1B (the major peak has a mass value of 39 148); (C) GSSG-treated PTP1B. Panels B and C show the deconvoluted spectra expressed as relative intensity versus mass.

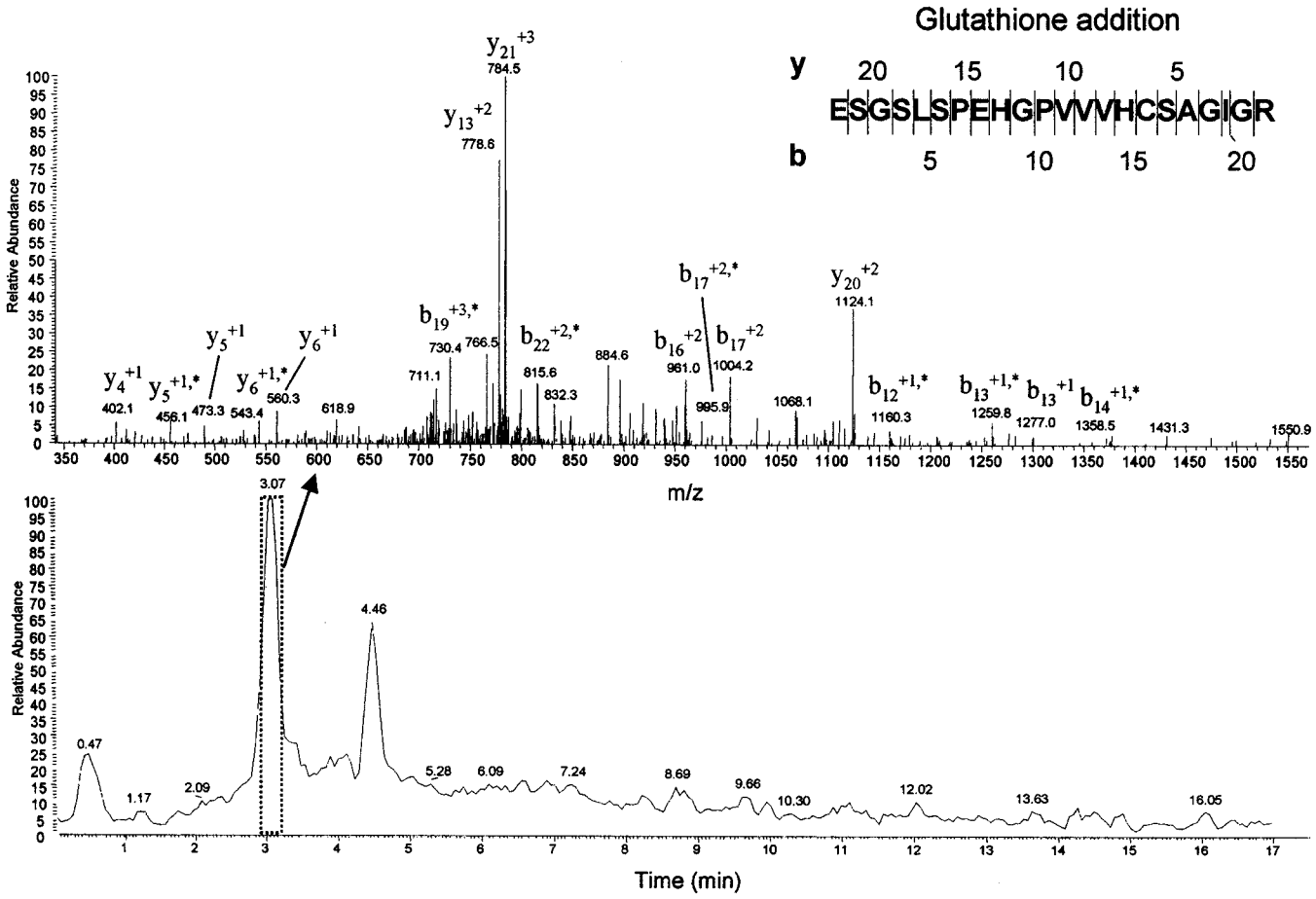

FIGURE 5: Quadrupole ion trap LC/MS/MS of tryptic fragment 28 of PTP-1B treated with 25 mM GSSG containing the active site Cys215. The bottom LC trace shows elution of fragment 28 (2479.05 Da) whose triply charged ion fragments as shown in the top trace.

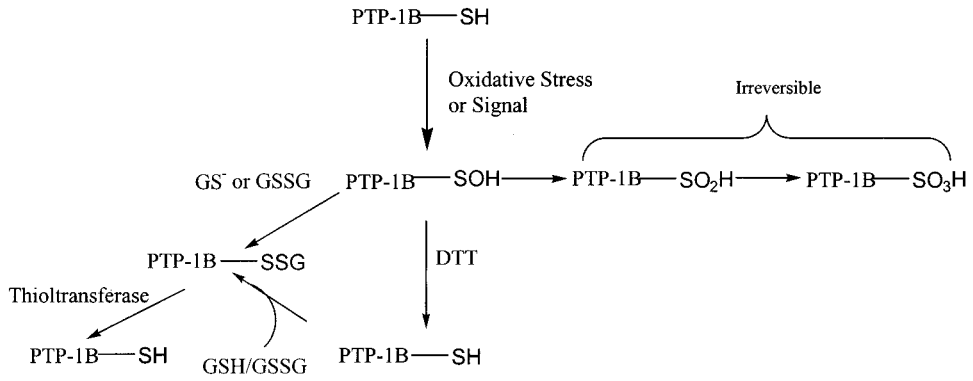

FIGURE 6: Proposed mechanism of glutathionylation of PTP1B to protect against excess oxidation, and constitute an efficient regulatory mechanism.

The mixed disulfide glutathionylated protein (GS−SP) is a much more stable inactive phosphatase than the cysteine sulfenic derivative which can undergo rapid irreversible inactivation to the cysteine sulfinic and cysteine sulfonic end products. The mechanism for redox regulation of PTP1B may be modified from that of Denu and Tanner (*9*) to include the additional step of glutathionylation (Figure 6), thus providing a stable derivative in highly oxidizing environments.

In a previous study by Lee et al. (*10*), isolated PTP1B was treated with H_2O_2, and the inactivated phosphatase was reactivated by the thioredoxin and thioltransferase (glutaredoxin) systems. The more rapid reactivation by thioredoxin compared to thioltransferase was interpreted to mean that the active site cysteine of PTP1B was oxidized to cysteine sulfenic acid by H_2O_2. In an analogous study, *in vitro* treatments of glyceraldehyde-3-phosphatse dehydrogenase (GAPD) with H_2O_2, and PFK with GSSG, were assumed to give GAPD-S-OH and PFK-SSG as the corresponding inactivated forms of the enzymes (*34*). Comparison of equimolar amounts of thioltransferase and thioredoxin near their physiological concentrations indicated that thioltransferase was much more effective than thioredoxin for reactivating PFK−SSG, as would be predicted from the substrate selectivity of thioltransferase delineated by Mieyal et al. (*19*, *20*). Thioredoxin, however, was better for reactivating the two types of modified GAPD (*34*).

A difficulty with extrapolating these studies (*10*, *34*) to the intracellular situation is that the inactivation of the

respective enzymes *in vitro* was carried out in the absence of GSH. This is especially problematical for interpreting the nature of the modified forms of PTP1B and GAPD because GSH is abundant in cells exposed to oxidizing agents. Inactivation of GAPD *in situ* has been correlated with glutathionylation (GAPD-SSG) in several cell types (*35*, *36*), and it is likely that PTP1B is also glutathionylated within cells. Whether PTP1B modification in situ is comprised of Cys-SOH and/or Cys-SSG remains to be investigated. However, the ability of GSH to convert Cys-SOH to Cys-SSG suggests that much of the modified enzyme inside the cell may be in the glutathionyl mixed disulfide form (PTP1B−SSG), which is reactivated preferentially by thioltransferase. Such a regulatory mechanism is consistent with the in vitro data reported in this study.

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