The Biological Buffer Bicarbonate/CO₂ Potentiates H₂O₂-Mediated Inactivation of Protein Tyrosine Phosphatases


ABSTRACT: Hydrogen peroxide is a cell signaling agent that inactivates protein tyrosine phosphatases (PTPs) via oxidation of their catalytic cysteine residue. PTPs are inactivated rapidly during H₂O₂-mediated cellular signal transduction processes, but, paradoxically, hydrogen peroxide is a rather sluggish PTP inactivator in vitro. Here we present evidence that the biological buffer bicarbonate/CO₂ potentiates the ability of H₂O₂ to inactivate PTPs. The results of biochemical experiments and high-resolution crystallographic analysis are consistent with a mechanism involving oxidation of the catalytic cysteine residue by peroxymonocarbonate generated via the reaction of H₂O₂ with HCO₃⁻/CO₂.

Hydrogen peroxide is a signaling agent that mediates cellular responses to growth factors, hormones, and cytokines such as platelet-derived growth factor, epidermal growth factor, vascular endothelial growth factor, insulin, tumor necrosis factor-α, and interleukin-1β. Protein tyrosine phosphatases (PTPs) are important targets of H₂O₂ produced during signal transduction processes. PTPs help regulate a variety of critical mammalian signaling pathways by catalyzing the removal of phosphoryl groups from phosphotyrosine residues on target proteins. H₂O₂ inactivates PTPs via oxidation of the catalytic cysteine residue in these enzymes, leading to elevated levels of tyrosine phosphorylation on key signaling proteins. Reactions with cellular thiols ultimately return oxidized PTPs to the catalytically active forms.

Peroxide-mediated inactivation of intracellular PTPs during signaling events typically occurs rapidly (5–15 min). Paradoxically, the rate constants measured for in vitro inactivation of purified PTPs by H₂O₂ (e.g., 10–40 M⁻¹ s⁻¹ for PTP1B) suggest that the loss of enzyme activity should be rather sluggish (½ = 5–200 h) at the low cellular concentrations of H₂O₂ thought to exist during signaling events (0.1–1 μM). This kinetic discrepancy led us and others to consider the possibility that H₂O₂ may undergo spontaneous or enzymatic conversion to more reactive oxidizing agents that effect rapid intracellular inactivation of PTPs.

Along these lines, we set out to explore a potential role for the biological bicarbonate/CO₂ buffer system in H₂O₂-mediated signal transduction. H₂O₂ reacts with bicarbonate/CO₂ to generate the highly reactive oxidant, peroxymonocarbonate (Figure 1a).

Figure 1. (a) Possible mechanism for the inactivation of PTP1B by H₂O₂–HCO₃⁻. (b) Treatment of PTP1B with H₂O₂–HCO₃⁻ yields the oxidized, sulfenyl amide form of the enzyme. The structure of the oxidized enzyme was solved at 1.7 Å resolution (pdb code 3SME). The image shows a simulated annealing omit map contoured at 3.0σ, covering Cys215 and flanking residues.

This process may be catalyzed by thiols and sulfides. Peroxymonocarbonate is an acyl peroxide, and on the basis of our recent studies of organic acyl peroxides, we anticipated that this species might be a potent PTP inactivator.

To address this question, we employed the catalytic domain (aa 1–322) of recombinant human PTP1B, as an archetypal member of the PTP family. First, we confirmed that H₂O₂ alone causes time-dependent inactivation of PTP1B with an apparent second-order rate constant of 24 ± 3 M⁻¹ s⁻¹ at 25 °C, pH 7. The extracellular and intracellular concentrations of bicarbonate are 25 and 14.4 mM, respectively, and we examined the effects of bicarbonate within this general concentration range. We found that the presence of potassium bicarbonate markedly increased the rate of time-dependent PTP1B inactivation by H₂O₂. For example, potassium bicarbonate increased the apparent second-order rate constants for inactivation of PTP1B by H₂O₂ to 202 ± 4 and 330 ± 11 M⁻¹ s⁻¹ at concentrations of 25 and 50 mM, respectively (25 °C, pH 7, Figure 2).

At physiological temperature (37 °C), the rate of inactivation by the H₂O₂–KHCO₃ system increases further to 396 ± 10 M⁻¹ s⁻¹ (KHCO₃ 5 mM, pH 7, Figure S9). Other bicarbonate salts (NaHCO₃ and NH₄HCO₃) produced similar effects (Figure S21). Preincubation of H₂O₂ and KHCO₃ (up to 2 h) prior to addition of the enzyme did not significantly alter the observed rate of inactivation (Figure S10). Control experiments showed that KCl (25 mM), NaCl (25 mM), and KHCO₃ (5 mM) had no effect on PTP1B.

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or MgCl$_2$ (2 mM) did not significantly alter the rate at which H$_2$O$_2$ inactivated PTP1B (Figures S11–S13). This indicates that the effect of bicarbonate on the peroxide-mediated inactivation of PTP1B was not merely an ionic strength effect. It is important to emphasize that KHCO$_3$ alone did not cause time-dependent inactivation of PTP1B at 24 °C (Figure 2a). The time-dependent nature of the inactivation observed here is consistent with a process involving covalent chemical modification of the enzyme.

The cellular milieu contains millimolar concentrations of thiols such as glutathione, which can decompose peroxides.$^{1}$ Therefore, we examined the effects of glutathione on the inactivation of PTP1B by H$_2$O$_2$–KHCO$_3$. We find that the H$_2$O$_2$–KHCO$_3$ system causes rapid and complete loss of enzyme activity in the presence of glutathione (1 mM), with only an approximately 2-fold decrease in the observed rate of inactivation (Figure 3a).

Time-dependent inactivation of PTP1B by the H$_2$O$_2$–KHCO$_3$ system was slowed by competitive inhibitors (Figures S14 and S15). For example, phosphate (50 mM) slowed inactivation by a factor of 1.7 ± 0.1. Activity did not return to the inactivated enzyme following gel filtration or dialysis to remove H$_2$O$_2$ and KHCO$_3$ (Figures S22 and S23). These results suggest that inactivation of PTP1B by H$_2$O$_2$–KHCO$_3$ involves covalent modification of an active-site residue. Catalytic activity was recovered upon treatment of the inactivated enzyme with thiols such as dithiothreitol (DTT, Figures 3b and S24). For example, when the enzyme was inactivated by treatment with H$_2$O$_2$–KHCO$_3$ (34 μM and 25 mM, respectively, for 3 min to yield 80% inactivation), almost all (98%) of the initial activity was recovered by treatment with DTT (50 mM, 30 min, 25 °C). The thiol-reversible nature of the inactivation reaction is consistent with a mechanism involving oxidation of the enzyme’s catalytic cysteine residue.$^{3,6}$ Indeed, crystallographic analysis of PTP1B treated with H$_2$O$_2$–KHCO$_3$ produced a 1.7 Å resolution structure showing that the catalytic cysteine residue was oxidized to the cyclic sulfenyl amide residue observed previously for this enzyme (Figure 1b and Supporting Information).$^{3,6–8}$ There was no evidence in the electron density map for oxidation at any other residue in the enzyme. Peroxyborate can decompose to yield highly reactive oxygen radicals in the presence of transition metals.$^{16,19}$ However, addition of radical scavengers or chelators of adventitious trace metals had no effect on the inactivation of PTP1B by the H$_2$O$_2$–KHCO$_3$ system (Figures S16–S18), suggesting that the enzyme inactivation process described here proceeds via a two-electron oxidation mechanism as shown in Figure 1a.

We also examined the ability of bicarbonate to potentiate H$_2$O$_2$-mediated inactivation of SHP-2, a different member of the PTP enzyme family. The intracellular activity of SHP-2 is thought to be redox regulated in response to platelet-derived growth factor, endothelin-1, and T-cell receptor stimulation.$^{1}$ Our experiments employed the catalytic domain (aa 246–527) of the recombinant human enzyme. We found that the inactivation of SHP-2 by H$_2$O$_2$ occurs with an apparent second-order rate constant of 15 ± 2 M$^{-1}$ s$^{-1}$ (25 °C, pH 7, Figure S19).$^{20}$ The presence of potassium bicarbonate (25 mM) increased the apparent second-order rate constant for inactivation of SHP-2 to 167 ± 12 M$^{-1}$ s$^{-1}$, an 11-fold rate increase (Figure S20). Similar to PTP1B, the inactivation process was slowed by the competitive inhibitor sodium phosphate, and enzyme activity was recovered by treatment of the inactivated enzyme with DTT (Figures S25 and S26).
Finally, for the purposes of comparison, we measured the ability of H₂O₂ alone, or the H₂O₂—KHCO₃ system, to inactivate a different type of cysteine-dependent enzyme, papain. We found that H₂O₂ alone inactivates this cysteine protease with an observed second-order rate constant of 43 ± 7 M⁻¹ s⁻¹, whereas inactivation of papain by the H₂O₂—KHCO₃ (25 mM) system occurs with an observed rate constant of 83 ± 9 M⁻¹ s⁻¹ (Figures S27 and S28). The ~2-fold enhancement in the rate of papain inactivation engendered by bicarbonate is modest compared to its effect on the oxidation of PTPs and is similar to the 2-fold enhancement exerted by bicarbonate (25 mM) on the observed rate of peroxide-mediated oxidation of low-molecular-weight thiols. The larger effect of bicarbonate on the H₂O₂-mediated inactivation of PTPs suggests that this may be a mechanism-based inactivation process, in which the anion-binding pocket and general acid—base residues at the active site of the enzyme catalyze oxidation of the active-site cysteine by H₂O₂—KHCO₃. In summary, we report that the biological buffer system bicarbonate/CO₂ selectively potentiates the ability of H₂O₂ to inactivate PTPs. Bicarbonate/CO₂ enables steady-state concentrations of H₂O₂ in the low micromolar range to inactivate PTPs biologically relevant time frame of 10–15 min. Still, the rate constants reported here do not rise to the levels reported for other cellular H₂O₂ sensors such as the peroxiredoxins. Therefore, spontaneous conversion of the second messenger H₂O₂ to peroxymonocarbonate may work in tandem with colocalization, compartmentalization, or other means of generating localized H₂O₂ concentration gradients to effect rapid, transient down-regulation of selected PTPs during signal transduction processes. The chemistry described here, in some regards, is reminiscent of the abilities of superoxide and CO₂ to modulate the properties of the cell signaling agent nitric oxide.

**REFERENCES**


Supporting Information

The Biological Buffer, Bicarbonate/CO₂, Potentiates H₂O₂-Mediated Inactivation of Protein Tyrosine Phosphatases

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Materials. Reagents were purchased from the following suppliers: sodium acetate, tris(hydroxymethyl)aminomethane (Tris), 2-[bis-(2-hydroxyethyl)-amino]-2-hydroxymethyl-propane-1,3-diol (Bis-tris), 4-nitrophenyl phosphate disodium salt hexahydrate (PNPP), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), L-glutathione reduced (GSH), D,L-dithiothreitol (DTT), ammonium bicarbonate, ethylenediaminetetraacetic acid, disodium salt dehydrate (EDTA), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), diethylenetriaminepentaacetic acid (DTPA), sodium hydrogen phosphate, papain (cat# P-3125), and Nα-benzoyl-L-arginine 4-nitroanilide hydrochloride (cat# B3279) were obtained from Sigma-Aldrich (St. Louis, MO). Sodium chloride, sodium hydroxide, potassium bicarbonate, sodium phosphate, and sodium bicarbonate were obtained from Fisher. Zeba mini and micro centrifugal buffer exchange columns, and Tween-80 were purchased from Pierce Biotechnology (Rockford, IL). Amicon Ultra centrifugal filter devices were purchased from Millipore (Milford, MA). The enzyme consisting of amino acids 1-322 of human PTP1B was expressed and purified as described previously1 and the concentration of active enzyme in stock solutions was determined as described by Pregel et al.2 “Thiol-free” PTP1B was prepared by two sequential Zeba mini centrifugal buffer exchange columns according to manufacturer protocol. The buffer exchange columns were equilibrated before use in sodium acetate (100 mM), Tris (50 mM), Bis-tris (50 mM, pH 7), DTPA (10 mM), and Tween 80 (0.05%). The thiol-free enzyme was further diluted, if necessary, with sodium acetate (100 mM), Tris (50 mM), Bis-tris (50 mM, pH 7), DTPA (10 mM), and Tween 80 (0.05%) to achieve final stock concentrations of 600 nM–8 μM active enzyme.
**PTP1B 1-321 and 1-298 constructs.** For the crystallographic studies, PTP1B catalytic domain constructs having cleavable polyhistidine tags were engineered. The coding sequence for residues 1-321 was subcloned into pKA8H vector using *NdeI* and *BamHI* site such that the *N*-terminal His$_8$ tag was cleavable using tobacco etch virus protease (TEVP). Briefly, the plasmid containing PTP1B coding sequence was amplified using PCR. The PCR product was gel purified using a 1% DNA agarose gel. The purified PCR product was ligated into pZErO vector at 16 °C. The resulting ligation product was transformed into DH5α and plated onto LB agar plates supplemented with 40 µg/mL kanamycin and the plate was incubated at 37 °C overnight. Following incubation, four single colonies were picked and grown in LB media supplemented with 40 µg/mL kanamycin. These cultures were incubated at 37 °C and 250 rpm overnight in an incubator-shaker and further used for plasmid preparation. The isolated plasmids were excised with *NdeI* and *BamHI* and gel purified as described above. These inserts were ligated into pKA8H vector (cut with *NdeI/BamHI* and purified) followed by transformation into DH5α. The transformants were plated on LB agar plates supplemented with 50µg/mL ampicillin. Four single colonies were picked again for plasmid preparation and the clone was verified by DNA sequencing.

A shorter version of PTP1B including residues 1-298 was created by inserting a stop codon into the aforementioned plasmid. The QuickChange kit (Stratagene) was used for this purpose, and the clone was confirmed by DNA sequencing.

**Expression and Purification of PTP1B (1-298 domain).** The PTP1B (1-298) plasmid was transformed into *E. coli* BL21AI cells and plated on LB Agar containing ampicillin (50 µg/mL). The plate was incubated at 37 °C overnight and a single colony
of the transformant was picked to inoculate 10 mL starter culture made of 1% tryptone and 0.5% yeast extract. This was incubated at 37 °C with constant shaking at 250 rpm, overnight. The starter culture was used to inoculate 1 L of auto-induction media, and the cells were allowed to shake constantly for two hours at 37 °C and 250 rpm. After two hours of cell growth, the temperature was reduced to 25 °C, and 0.2 % arabinose was added to the media. Cells were harvested after 20-21 hours by centrifugation at 4 °C and 3500 rpm and resuspended in Buffer A (20 mM Tris, 150 mM NaCl, 10% glycerol pH 7.5). The cell pellet was quick-frozen into liquid nitrogen for later use.

Frozen cells were thawed at 4 °C in the presence of the following protease inhibitors: 10 µM leupeptin, 1 µM pepstatin A, 1 mM PMSF. Cells were stirred for 15-20 minutes at 4 °C followed by disruption using sonication. Unbroken cells and debris were removed by centrifugation for 60 min at 17,000 rpm. The supernatant was collected and subjected to a second centrifugation step for 30 min at 17,000 rpm. The resulting supernatant was used for further purification by immobilized metal-ion affinity chromatography (Ni\(^{2+}\)-charged HiTRAP; GE Healthcare). The fractions were eluted using buffer B (Buffer A supplemented with 1 M imidazole). Fractions containing PTP1B were pooled and mixed with TEVP (1 mg of TEVP per 40 mg of PTP1B) and 1 mM THP. The sample was incubated for 8 h at 20 °C and then dialyzed against buffer A. The dialyzed protein was again loaded onto the Ni\(^{2+}\) charged column using buffer A. Tag-free PTP1B was collected in both the flow-through and by elution in 3% buffer B. The purified protein was dialyzed into 10 mM Tris, 25 mM NaCl, 1 mM EDTA, 1 mM THP pH 7.5. Finally, the protein was distributed into thin-walled PCR tubes, quick-frozen in liquid nitrogen, and stored at –80 °C.
**SHP2 expression and purification.** The SHP2 encoding plasmid was transformed into BL21AI cells and plated on LB–Agar supplemented with kanamycin (40 µg/ml). The plate was incubated at 37 ºC overnight and a single colony of the transformant was picked to inoculate a 10 mL starter culture made of 1% tryptone and 0.5% yeast extract. This was incubated at 37 ºC with constant shaking at 250 rpm, overnight. The starter culture was used to inoculate 1 L of autoinduction media and cells were allowed to shake constantly for 2 h at 37 ºC and 250 rpm. After 2 h of cell growth, the temperature was reduced to 18 ºC and 0.2 % arabinose was added to the media. Cells were harvested after 28 h at 4 ºC and 3500 rpm by centrifugation and resuspended in 10 mM HEPES, 250 mM NaCl pH 7.5. The cell pellet was flash frozen in liquid nitrogen and was stored at −80 ºC.

Frozen cells were thawed at 4 ºC and ruptured using a sonicator. Unbroken cells and debris was removed by centrifugation for 60 min at 17,000 rpm. The supernatant was collected and subjected to a second centrifugation step for 30 min at 17,000 rpm. The resulting supernatant was used for further purification by immobilized metal-ion affinity chromatography (Ni²⁺-charged HiTRAP; GE Healthcare), followed by anion-exchange chromatography (HiTRAP Q; GE Healthcare). The purified enzyme was dialyzed into 10 mM HEPES, 250 mM NaCl, and 1 mM DTT at pH 7.5.

**Supplemental Method 1. Enzyme Inactivation Assays.** Thiol-free PTP1B was added to a mixture containing various concentrations of H₂O₂, various concentrations of KHCO₃ and buffer to achieve final concentrations of PTP1B (0.3 µM), sodium acetate (100 mM), Tris (50 mM), Bis-tris (50 mM, pH 7), DTPA (5 mM), and Tween 80
After mixing, the reactions were incubated at 25 °C. Assays without potassium bicarbonate employed 125-750 µM H₂O₂; assays containing 2 and 3.5 mM potassium bicarbonate employed 62.5-375 µM H₂O₂; assays containing 7 mM potassium bicarbonate employed 35-214 µM H₂O₂; assays containing 14.4 mM potassium bicarbonate employed 19-115 µM H₂O₂; assays containing 25 mM potassium bicarbonate employed 11-68 µM H₂O₂; assays containing 35 mM potassium bicarbonate employed 7-46 µM H₂O₂; assays containing 50 mM potassium bicarbonate employed 6-37 µM H₂O₂. The final pH of the inactivation mixtures did not vary more than ± 0.2 units from that of the starting buffer. Minor variations in pH cannot account for the bicarbonate effects reported here, as previous work shows that minor pH variations in the range 6.5-8.0 do not substantially alter the rate at which H₂O₂ alone (no bicarbonate) inactivates a PTP enzyme (see Figure 4 in ref 26). Enzyme activity remaining at various time points (30 s, 60 s, 90 s, 120 s, 150 s, and 180 s) was assessed by a method similar to those described previously. Specifically, an aliquot (10 µL) of the inactivation reaction into an assay mixture (490 µL) containing Bis-tris (50 mM, pH 6.0), NaCl (100 mM), DTPA (10 mM), and p-nitrophenyl phosphate (p-NPP, 20 mM), followed by incubation at 30 °C for 10 min. The activity assay was quenched by addition of NaOH (500 µL of a 2 N solution in DI water) and the amount of p-nitrophenol released during the assay determined by measurement of the absorbance of p-nitrophenolate at 410 nm at 24 °C. Inactivation reactions including additives such as tryptophan (1 mM), sodium phosphate (50 mM), mannitol (100 mM), or desferal (1 mM) were performed as described above and the additives were present in the inactivation mixture prior to addition of the enzyme.
Inactivation assays involving SHP-2 were carried out in an identical manner except somewhat lower enzyme concentrations were employed (~ 0.2 µM).

The value of ln(A/A₀), where A is the enzyme activity remaining at time = t and A₀ is the enzyme activity at time = 0, was plotted against t and the pseudo-first-order rate constant for inactivation at each concentration of H₂O₂ (kₐₙₙ) calculated from the slope of the line. The apparent second-order rate constant for the inactivation process was obtained from the slope of a replot of kₐₙₙ versus H₂O₂ concentration.⁴,⁶ The results of enzyme inactivations carried out under various conditions are shown below in Figures S1-S20. To investigate the stability of bicarbonate solutions under our reaction conditions, we added basic BaCl₂ to a mock assay solution and measured the resulting BaCO₃ precipitate.⁷ This analysis revealed that the solutions retained the desired bicarbonate concentration over the course of a typical assay.

**Figure S1.** Inactivation of PTP1B by H₂O₂ alone. The lines correspond to 0, 125, 250, 375, 500, 625, 750 µM H₂O₂ from top to bottom. kₐₙₙ = 24 ± 3 M⁻¹ s⁻¹.
**Figure S2.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (2 mM). The lines correspond to 0, 62, 125, 188, 250, 312, 375 µM H$_2$O$_2$ from top to bottom. $k_{\text{app}} = 33 \pm 5$ M$^{-1}$ s$^{-1}$.

**Figure S3.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (3.5 mM). The lines correspond to 0, 62, 125, 188, 250, 312, 375 µM H$_2$O$_2$ from top to bottom. $k_{\text{app}} = 61 \pm 2$ M$^{-1}$ s$^{-1}$.
**Figure S4.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (7 mM). The lines correspond to 0, 36, 71, 107, 143, 179, 214 µM H$_2$O$_2$ from top to bottom. $k_{app} = 74 \pm 14$ M$^{-1}$ s$^{-1}$.

**Figure S5.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (14.4 mM). The lines correspond to 0, 19, 38, 58, 77, 96, 115 µM H$_2$O$_2$ top to bottom. $k_{app} = 117 \pm 11$ M$^{-1}$ s$^{-1}$.
**Figure S6.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 µM H$_2$O$_2$ from top to bottom. $k_{app} = 202 \pm 4$ M$^{-1}$ s$^{-1}$.

**Figure S7.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (35 mM). The lines correspond to 0, 8, 16, 23, 31, 39, 47 µM H$_2$O$_2$ from top to bottom. $k_{app} = 274 \pm 15$ M$^{-1}$ s$^{-1}$.
Figure S8. Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (50 mM). The lines correspond to 0, 6, 12, 19, 25, 31, 38 µM H$_2$O$_2$ from top to bottom. k$_{app}$ = 330 ± 11 M$^{-1}$ s$^{-1}$

Figure S9. Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) at 37 °C (rather than 25 °C which was used for the assays shown above). The lines correspond to 0, 10, 21, 31, 42, 52, 62 µM H$_2$O$_2$ from top to bottom. k$_{app}$ = 396 ± 10 M$^{-1}$ s$^{-1}$. At 37 °C, in the presence of bicarbonate salts (NaHCO$_3$), a slow inactivation of the enzyme occurring with an apparent rate constant of 0.04 M$^{-1}$ s$^{-1}$ is observed even in the absence of H$_2$O$_2$. 
Figure S10. Preincubation of H$_2$O$_2$-KHCO$_3$ (25 mM) for 2 h prior to addition of PTP1B does not significantly change inactivation rates, relative to assays without preincubation. The lines correspond to 0, 11, 23, 34, 45, 57, 68 µM H$_2$O$_2$ from top to bottom. $k_{app} = 199 \pm 7$ M$^{-1}$ s$^{-1}$ (compare to Figure S6, H$_2$O$_2$-KHCO$_3$ (25 mM) without preincubation, $k_{app} = 202 \pm 4$ M$^{-1}$ s$^{-1}$).

Figure S11. Inactivation of PTP1B by H$_2$O$_2$ in presence of sodium chloride (25 mM). The lines correspond to 0, 125, 250, 375, 500, 625, 750 µM H$_2$O$_2$ from top to bottom. $k_{app} = 23 \pm 4$ M$^{-1}$ s$^{-1}$ (compare to inactivation of PTP1B by H$_2$O$_2$ without added NaCl, Figure S1, $k_{app} = 24 \pm 3$ M$^{-1}$ s$^{-1}$).
**Figure S12.** Inactivation of PTP1B by H₂O₂ in presence of potassium chloride (25 mM). The lines correspond to 0, 125, 250, 375, 500, 625, 750 µM H₂O₂ from top to bottom. \( k_{app} = 25 \pm 4 \text{ M}^{-1} \text{s}^{-1} \) (compare to inactivation of PTP1B by H₂O₂ without added KCl, Figure S1, \( k_{app} = 24 \pm 3 \text{ M}^{-1} \text{s}^{-1} \)).

**Figure S13.** Inactivation of PTP1B by H₂O₂ in presence of magnesium chloride (2 mM). The lines correspond to 0, 125, 250, 375, 500, 625, 750 µM H₂O₂ from top to bottom. \( k_{app} = 24 \pm 5 \text{ M}^{-1} \text{s}^{-1} \) (compare to inactivation of PTP1B by H₂O₂ without added MgCl₂, Figure S1, \( k_{app} = 24 \pm 3 \text{ M}^{-1} \text{s}^{-1} \)).
Figure S14. Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) in presence of the competitive inhibitor sodium phosphate (50 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 µM H$_2$O$_2$ from top to bottom. $k_{app} = 117 \pm 10$ M$^{-1}$s$^{-1}$ (compare to inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) without added sodium phosphate, Figure S6, $k_{app} = 202 \pm 4$ M$^{-1}$s$^{-1}$).

Figure S15. Inactivation of PTP1B (1.2 µM) by H$_2$O$_2$-KHCO$_3$ (60 µM-25 mM) in the presence and absence of the competitive inhibitor, S1 (50 nM). Enzyme activity was measured as described. The upper points (red squares) depict the time course for the enzyme treated with H$_2$O$_2$-KHCO$_3$ (60 µM/25 mM) in the presence of S1 (50 nM). The lower points (blue diamonds) depict the time course for the enzyme treated with H$_2$O$_2$-KHCO$_3$ (60 µM/25 mM) without S1. The IC$_{50}$ of S1 against PTP1B is 47 nM.
In principle, the inactivation of PTP1B by peroxides may proceed by either one-electron or two-electron oxidation mechanisms.\(^9,10\) A one-electron process would involve metal-mediated reduction of the peroxide bond to yield a highly reactive oxygen radical.\(^9,10\) In the present case, a radical-mediated inactivation process seemed unlikely given that the reactions were performed in a radical-scavenging buffer (Tris/bis-Tris) that contained a chelator (diethylenetriamine pentaacetic acid) that inhibits metal-dependent conversion of peroxides to oxygen-centered radicals.\(^9\) Nonetheless, we examined this issue and found that the presence of an additional trace metal chelator, desferal (1 mM) or radical scavengers such as mannitol (100 mM) and tryptophan (1 mM) had no significant effect on the inactivation of PTP1B by hydrogen peroxide in the presence of bicarbonate (Figures S16-S18). These results are consistent with the involvement of peroxymonocarbonate in the enzyme inactivation process, as this agent has previously been shown to act as a two-electron oxidant of sulfhydryl groups.\(^11\)
**Figure S16.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) in presence of desferal (1 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 µM H$_2$O$_2$ from top to bottom. $k_{app} = 215 \pm 14$ M$^{-1}$s$^{-1}$ (compare to inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) without added desferal, Figure S6, $k_{app} = 202 \pm 4$ M$^{-1}$s$^{-1}$).

**Figure S17.** Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) in presence of mannitol (100 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 µM H$_2$O$_2$ from top to bottom. $k_{app} = 184 \pm 18$ M$^{-1}$s$^{-1}$ (compare to inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) without added mannitol, Figure S6, $k_{app} = 202 \pm 4$ M$^{-1}$s$^{-1}$).
Figure S18. Inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) in presence of tryptophan (1 mM). The lines correspond to 0, 11, 23, 34, 45, 68 µM H$_2$O$_2$ from top to bottom. $k_{app} = 244 \pm 23$ M$^{-1}$ s$^{-1}$ (compare to inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM) without added tryptophan, Figure S6, $k_{app} = 202 \pm 4$ M$^{-1}$ s$^{-1}$).

Figure S19. Inactivation of SHP-2 by H$_2$O$_2$. The lines correspond to 0, 375, 500, 625, 750, 875, 1000 µM H$_2$O$_2$ from top to bottom. $k_{app} = 15 \pm 2$ M$^{-1}$ s$^{-1}$. 
Figure S20. Inactivation of SHP-2 by H₂O₂ in presence of potassium bicarbonate (25 mM). The lines correspond to 0, 20, 35, 50, 65, 80, 95 µM H₂O₂ from top to bottom. \( k_{\text{app}} = 167 \pm 12 \, \text{M}^{-1} \text{s}^{-1} \).

Supplemental Method 2. Continuous Assay for Inactivation of PTP1B by H₂O₂ and Various Bicarbonate Salts. Thiol-free PTP1B was added to a cuvette containing substrate, buffer, bicarbonate, and H₂O₂ to achieve final concentrations of p-nitrophenyl phosphate (10 mM), sodium acetate (100 mM), Bis-Tris (50 mM), Tris (50 mM), bicarbonate salt (14.4 or 25 mM), and H₂O₂ (200 µM). Immediately following addition of enzyme to the cuvette, the reaction was mixed by inversion (3x), and enzyme-catalyzed release of p-nitrophenol was monitored at 410 nm, and 25 °C. Data points were taken every 2 s. The results of these assays are shown in Figure S21, below.
**Figure S21.** Comparison of the ability of KHCO$_3$, NaHCO$_3$, and NH$_4$HCO$_3$ to enhance the inactivation of PTP1B by H$_2$O$_2$. **Left side:** Control enzyme (no H$_2$O$_2$ or HCO$_3^-$, upper, blue line), inactivation of PTP1B by H$_2$O$_2$-NaHCO$_3$ (14.4 mM, upper, teal green curve), inactivation of PTP1B by H$_2$O$_2$-NH$_4$HCO$_3$ (14.4 mM, red curve), and inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (14.4 mM, bottom, dark green curve). **Right side:** Control enzyme (no H$_2$O$_2$ or HCO$_3^-$, upper, blue line), inactivation of PTP1B by H$_2$O$_2$-NaHCO$_3$ (25 mM, upper, teal green curve), inactivation of PTP1B by H$_2$O$_2$-NH$_4$HCO$_3$ (25 mM, red curve), and inactivation of PTP1B by H$_2$O$_2$-KHCO$_3$ (25 mM, bottom, dark green curve). Inactivation by H$_2$O$_2$-NH$_4$HCO$_3$ and H$_2$O$_2$-KHCO$_3$ are comparable. Inactivation by NaHCO$_3$ is less effective, perhaps due to the lower solubility of this bicarbonate salt.

**Supplemental Method 3. Gel Filtration of PTP1B Inactivated by H$_2$O$_2$-KHCO$_3$.**

Thiol free PTP1B (0.35 µM) was incubated for 3 min at 24 °C with H$_2$O$_2$ (70 µM) and KHCO$_3$ (25 mM) in a mixture containing NaOAc (100 mM), Bis-Tris (50 mM), Tris (50 mM), Surfact-Amps® 80 (0.025% v/v), DTPA (5 mM) pH 7.0. An aliquot was removed from this solution and the remaining enzyme activity was measured as described above. The remainder of the solution was subjected to buffer exchange via centrifugal gel filtration using a Zeba micro spin column according to manufacturer protocol. Exchange buffer contained: sodium acetate (100 mM), Tris-HCl (50 mM), Bis-tris (50 mM), DTPA (10 mM), Surfact-Amps® 80 (0.05 % v/v) at pH 7. Following buffer exchange, the resulting enzyme-containing filtrate was assayed for PTP activity as described above. The results are shown in Figure S22, below.
Figure S22. Activity of H$_2$O$_2$-KHCO$_3$-inactivated PTP1B does not return following gel filtration. The first and second bars show the activity of control, untreated enzyme before and after gel filtration. The second two bars show the “activity” of inactivated enzyme before and after gel filtration.

Supplemental Method 4. Dialysis of PTP1B Inactivated by H$_2$O$_2$-KHCO$_3$. Thiol free PTP1B (0.35 µM) was incubated for 3 min at 24 °C with H$_2$O$_2$ (70 µM) and KHCO$_3$ (25 mM) in a mixture containing sodium acetate (100 mM), Bis-Tris (50 mM), Tris (50 mM), Surfact-Amps® 80 (0.025% v/v), DTPA (5 mM) pH 7.0. An aliquot was removed from this solution and the remaining enzyme activity was measured as described above. The remainder of the solution was loaded onto a Slide-A-Lyzer MINI Dialysis Unit and dialyzed for 100 min at 4 °C against dialysis buffer consisting of Tris (50 mM), Bis-Tris (50 mM), NaOAc (100 mM), DTPA (10 mM), and Surfact-Amps® 80 (0.05% v/v) detergent at pH 7.0. Following dialysis, the solution inside the dialysis chamber was assayed for activity as described above. The results are shown in Figure S23, below.
Figure S23. Activity of H₂O₂-KHCO₃-inactivated PTP1B does not return following dialysis. The first and second bars show the activity of control, untreated enzyme before and after dialysis. The second two bars show the “activity” of inactivated enzyme before and after dialysis.

Supplemental Method 5. Treatment of PTP1B Inactivated by H₂O₂ or H₂O₂-KHCO₃ with Dithiothreitol (DTT). Thiol free PTP1B (0.64 µM) was incubated for 3 min at 24 °C with H₂O₂ (375 µM) in a mixture containing NaOAc (100 mM), Bis-Tris (50 mM), Tris (50 mM), Surfact-Amps® 80 (0.025% v/v), DTPA (5 mM) pH 7.0. An aliquot was removed from this solution and the remaining enzyme activity was measured as described above. To the remainder of the solution was added DTT to a final concentration of 50 mM. The resulting mixture was incubated at 25 °C for 30 min and then assayed for PTP activity as described above. The results of this assay are shown below in Figure S24.
**Figure S24.** Treatment with DTT leads to the recovery of activity of oxidatively-inactivated PTP1B. In each plot, the first and second bars correspond to the activity of control, untreated enzyme and inactivated enzyme, respectively (no DTT treatment). The second two bars show the activity of control, untreated enzyme and inactivated enzyme, following treatment with DTT as described above.

**Supplemental Method 6. Catalytic activity of SHP-2 inactivated by H₂O₂-KHCO₃ can be recovered by treatment with dithiothreitol (DTT).** Thiol-free SHP-2 (0.45 μM) was inactivated by treatment with H₂O₂ (150 μM) and KHCO₃ (25 mM) in assay buffer containing sodium acetate (100 mM), bis-Tris (50 mM, pH 7.0), Tris (50 mM), DTPA (50 μM), Tween-80 (0.00025%), and p-NPP (10 mM, 1 mL final volume). The reaction was carried out in a quartz cuvette and the enzyme activity was continuously monitored by following the release of p-nitrophenol at 410 nm. When enzyme inactivation was complete, as indicated by a plateau in the production of p-nitrophenol, DTT (5 µL of a 1 M in H₂O, to yield a final concentration of 5 mM DTT) was added to the cuvette using a long pipette tip, the solution mixed by pumping the pipette three
times, and the recovery of enzyme activity observed by monitoring the absorbance increase at 410 nm. The results are shown below in Figure S25, below.

**Figure S25.** Catalytic activity of SHP-2 inactivated by H$_2$O$_2$-KHCO$_3$ can be recovered by treatment with dithiothreitol (DTT).

**Figure S26.** Inactivation of SHP-2 by H$_2$O$_2$-KHCO$_3$ (430 µM/25 mM) in presence of the competitive inhibitor sodium phosphate (50 mM). Enzyme activity was measured as described above in Supplemental Methods 1. The upper points (blue diamonds) depict the loss of activity in enzyme treated with H$_2$O$_2$-KHCO$_3$ (430 µM/25 mM) in the presence of sodium phosphate (50 mM). The lower points (red squares) depict the loss of activity in enzyme treated with H$_2$O$_2$-KHCO$_3$ (430 µM/25 mM) *without* sodium phosphate.

**Supplemental Method 7. Inactivation of the Cysteine Protease Papain by H$_2$O$_2$ and H$_2$O$_2$-KHCO$_3$.** Papain (10 mg/mL) was activated by incubation with DTT (2 mM) for
30 min at 25 °C in sodium phosphate buffer (50 mM, pH 7) containing EDTA (2.5 mM) as described previously.\textsuperscript{12} Thiol was removed from the activated enzyme as described for PTP1B in the Materials Section. The buffer exchange columns were equilibrated with sodium phosphate (50 mM, pH 7), EDTA (2.5 mM). The resulting “thiol-free” papain was used immediately in inactivation assays. Typical inactivation assays contained various concentrations of H\textsubscript{2}O\textsubscript{2} either with, or without, KHCO\textsubscript{3} (25 mM) along with papain (1 mg/mL), sodium phosphate (50 mM, pH 7), EDTA (2.5 mM). At various times an aliquot (100 µL) of the inactivation reaction was transferred into a cuvette containing substrate solution (900 µL) to give final concentrations of \textit{N}\textsuperscript{α}-benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA,\textsuperscript{13} 1 mM), sodium phosphate (45 mM), EDTA (2.25 mM), and DMSO (10% v/v). Enzyme activity was measured by monitoring the initial rate of release of 4-nitroaniline at 410 nm (first 30 s). The percent remaining activity was calculated based on comparison to a control assay containing no H\textsubscript{2}O\textsubscript{2} or KHCO\textsubscript{3}. The results are shown below in Figures S27 and S28.

\textbf{Figure S27.} Inactivation of Papain by H\textsubscript{2}O\textsubscript{2} alone. The lines correspond to 0, 10, 20, 40, 60, 80 µM H\textsubscript{2}O\textsubscript{2} from top to bottom. \(k_{\text{app}} = 43 \pm 7 \text{ M}^{-1} \text{s}^{-1}\). This value is comparable to
published literature values for the inactivation of cysteine proteases by H_2O_2 in the absence of substrate.\textsuperscript{14,15}

![Graph](image)

**Figure S28.** Inactivation of Papain by H_2O_2-KHCO_3 (25 mM). The lines correspond to 0, 10, 15, 20, 30 µM H_2O_2 from top to bottom. \( k_{\text{app}} = 83 \pm 9 \text{ M}^{-1} \text{s}^{-1} \).

**Supplemental Methods 8. Crystal structure determination.** Crystallization trials were performed at 4º C using sitting drop vapor diffusion and a 10 mg/mL stock solution of PTP1B (1-298). Diffraction quality crystals were grown using 11-18 % PEG3000, 0.1 M HEPES pH 7.0-8.0, 0.2 M magnesium acetate, and 2 mM TCEP as reported previously.\textsuperscript{16}

Crystals of inactivated PTP1B were prepared by soaking PTP1B crystals at room temperature. The crystals were first cryoprotected using 20% PEG3000, 0.1 M HEPES pH 7.0, 0.2 M magnesium acetate, and 20% PEG 200, and then transferred to a solution containing the cryobuffer supplemented with 25 mM KHCO_3 and 50 µM H_2O_2. The soak time was varied from 20 to 45 min. Crystals were picked up with Hampton loops and plunged into liquid nitrogen.

A 1.7 Å resolution X-ray diffraction data set was collected at ALS beamline 4.2.2
and processed using d*TREK (Table S1). The crystals have space group $P3_121$ with unit cell dimensions of $a = 88.4$ Å and $c = 104.3$ Å; there is 1 molecule in the asymmetric unit. Molecular replacement phasing as implemented in PHENIX AutoMR was used. The search model was derived from a native PTP1B structure (PDB code 2f71) with following residues omitted: 46-49, 180-188, and 215-221. Note that this list includes the active site Cys215 and flexible active site loops whose conformations are sensitive to the oxidation state of Cys215 and the presence of bound ligands. The model from molecular replacement was improved with iterative rounds of model building in Coot and refinement in PHENIX.

The 1.7 Å resolution electron density map clearly showed the formation of the cyclic sulfenyl amide involving Cys215 and Ser216 (Figure S29). The conformations of the P-loop and other flexible active site loops are identical to those described previously for crystals soaked in H$_2$O$_2$. For example, electron density for the P-loop is shown in Figure S29.
### Table S1. X-ray data collection and refinement statistics$^a$

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$^a$Values for the outer resolution shell of data are given in parenthesis.
$^b$5% random test set.
$^c$Compared to the parameters of Engh and Huber.$^{24}$
$^d$The Ramachandran plot was generated with RAMPAGE.$^{25}$
$^e$Maximum likelihood-based coordinate error estimate.
**Figure S29.** Stereographic view of PTP inactivated by 25 mM KHCO₃ and 50 μM H₂O₂ (gray) or H₂O₂ alone (yellow, PDB code 1OEM). The P-loop is shown in sticks (residues 214-221). The cage represents a simulated annealing σₐ-weighted Fₘ-Fₑ omit map contoured at 3.0σ. Prior to map calculation, the P-loop was omitted and simulated annealing refinement was performed using PHENIX.

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Supplemental Literature Citations

(7) Harris, D. C. *Quantitative chemical analysis*; Freeman: San Francisco, 1982.