Bioorganic & Medicinal Chemistry Letters 18 (2008) 5856–5859

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Oxidative inactivation of protein tyrosine phosphatase 1B by organic hydroperoxides

Sanjib Bhattacharya, Jason N. LaButti, Derrick R. Seiner, Kent S. Gates *

Departments of Chemistry and Biochemistry, University of Missouri-Columbia, Columbia, MO 65211, USA

ARTICLE INFO

Article history:
Received 15 April 2008
Revised 10 June 2008
Accepted 10 June 2008
Available online 13 June 2008

Keywords:
Protein tyrosine phosphatase
Peroxides
Hydroperoxides
Enzyme inactivation

ABSTRACT

Protein tyrosine phosphatases (PTPs) are cysteine-dependent enzymes that play a central role in cell signaling. Organic hydroperoxides cause thiol-reversible, oxidative inactivation of PTP1B in a manner that mirrors the endogenous signaling agent hydrogen peroxide.

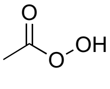
Protein tyrosine phosphatases (PTPs) are cysteine-dependent enzymes that catalyze the hydrolytic removal of phosphate groups from tyrosine residues in proteins (Scheme 1).[1–3] Thus, PTPs, in concert with protein tyrosine kinases, play a central role in cell signaling by regulating the phosphorylation status and, in turn, the functional properties of target proteins in various signal transduction pathways.[4,5]

The cellular activity of some PTPs is regulated by hydrogen peroxide (H_2O_2) that is produced as a second messenger in response to extracellular stimuli such as insulin, epidermal growth factor, and platelet-derived growth factor.[6–9] H_2O_2 inactivates PTPs via oxidation of the active site cysteine thiol residue to a sulfenic acid.[6,10] In some cases, the cysteine sulfenic acid undergoes subsequent conversion to an active site sulfenyl amide linkage[11–13] or disulfide.[14,15] This type of oxidative inactivation of PTPs is slowly reversed upon reaction of the inactivated enzyme with biological thiols (Scheme 1).[10–15]

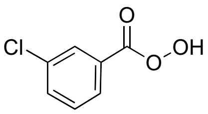
Given the biological importance of PTPs, it may be useful to identify organic reagents that regulate PTP activity through redox mechanisms.[16–20] With this in mind, we examined the ability of several organic hydroperoxides (**1**–**4**) to effect thiol-reversible, oxidative inactivation of the archetypal member of the PTP family, PTP1B.[21] We find that peracetic acid (**1**) is a potent, time-dependent inactivator of the catalytic subunit of PTP1B (aa 1–322) (Fig. 1A). The observation of time-dependent inactivation is consistent with a process involving covalent modification of the enzyme.[22] A good linear fit is obtained in a plot of the apparent rates of inactivation versus concentration, indicating that the inactivation reaction is a second-order process (Fig. 1B). The slope of the line reveals a rate constant of $2.1 \pm 0.2 \times 10^4$ M^{-1} s^{-1} for the reaction of **1** with PTP1B. For comparison, hydrogen peroxide inactivates PTP1B with a rate constant of 10 M^{-1} s^{-1}.[10]

The inactivation of PTP1B (250 nM) by **1** (1 μM, 3 min) is not reversed upon removal of excess inactivator by gel filtration of the enzyme through G25 Sephadex. The inactivation process is significantly slowed in the presence of phosphate ion, which is an active site-directed inhibitor of the enzyme (K_i = 17 mM). Specifically, treatment of the enzyme with **1** (15 μM) for 15 s inactivates 88% of the enzyme under standard conditions, while in the presence of phosphate ion (50 mM) only 49% inactivation is observed. Together, the results suggest that **1** inactivates PTP1B via covalent modification of the active site.

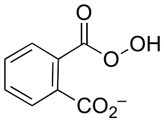
Inactivation of PTP1B by **1** can be reversed by treatment of the enzyme with thiols (Fig. 2). Specifically, inactivation of PTP1B



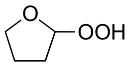
1



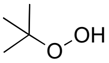
2



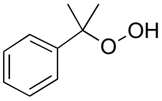
3



4



5

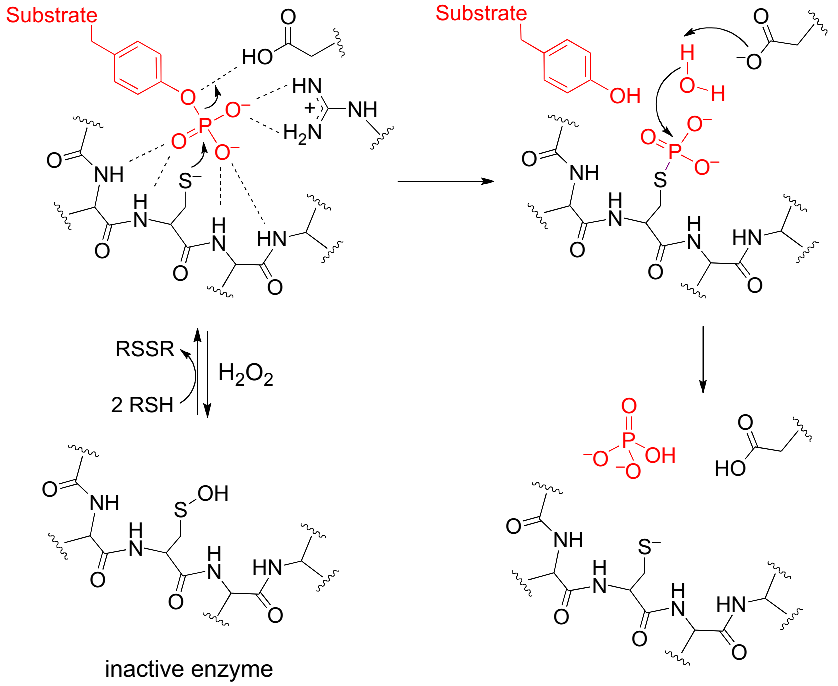


6

---

* Corresponding author. Tel.: +1 573 882 6763; fax: +1 573 882 2754.
E-mail address: gatesk@missouri.edu (K.S. Gates).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.029



Scheme 1.

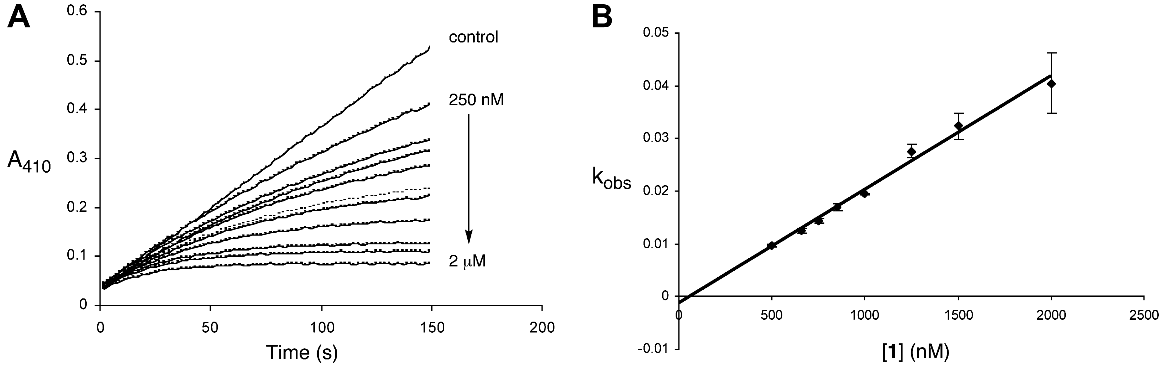


Figure 1. Rate of inactivation of PTP1B by compound **1**. (A) Progress curves for the inactivation of PTP1B by **1**. PTP1B was isolated as described previously[19,20] and thiol-free samples of the enzyme were prepared by gel filtration of the protein through G25 Sephadex immediately prior to use according to reported procedures.[19,20] Thiol-free PTP1B (25 nM final) was added to a solution of **1** (250 nM–2 μM) in 3,3-dimethyl glutarate buffer (50 mM, pH 7.0) containing the substrate *p*-nitrophenyl phosphate (*p*-NPP, 20 mM) at 23 °C. The apparent rate constant (k_{obs}) for each concentration was calculated by the method of Voet and co-workers.[23] (B) Determination of the second-order rate constant for the inactivation of PTP1B by **1**. The apparent (pseudo-first-order) rate constants for inactivation of PTP1B by **1** were plotted versus concentration and the second-order rate constant for inactivation of PTP1B by **1** was extracted from the slope. Inactivator concentrations >10-times the enzyme concentration were used in the plot. The concentration of **1** (and other peroxides) in stock solutions was determined by titration.[24]

(750 nM) with low concentrations of **1** (800 nM, 10 min, 23 °C), followed by treatment of the inactive enzyme with dithiothreitol (DTT, 100 mM final concentration), led to substantial return of enzymatic activity (73% of initial activity). (When enzyme is not treated by **1**, essentially all activity is retained under these conditions). This suggests that reaction of PTP1B with **1** predominantly converted the active site cysteine residue to the sulfenic acid oxidation state. In contrast, following treatment of the enzyme with higher concentrations of **1** (5 μM, 10 min, 23 °C), only 10% of the original activity returned upon treatment with DTT. Under these conditions, the active site cysteine presumably underwent significant amounts of 'over-oxidation' to the sulfinic (RSO_2H) or sulfonic acid (RSO_3H) oxidation states that are not readily reduced to active enzyme by DTT. It is known that cysteine residues in redox regulated enzymes can be irreversibly 'over-oxidized' to sulfinic and sulfonic acids by hydrogen peroxide under some conditions.[12,14,25]

Two possible mechanisms can be considered for the inactivation of PTP1B by **1**. Direct reaction of the active site cysteine residue with **1** would yield the inactive, sulfenic acid form of the enzyme (Scheme 2A).[26–28] Alternatively, a metal-mediated Fenton-type reaction[29] could generate diffusible oxygen radicals that might oxidize the active site cysteine residue.[30] Two lines of experimentation argue against a Fenton-type process in the inactivation of PTP1B by **1**. First, we find that addition of the classical oxygen radical scavenging agent mannitol[29] has little effect on the inactivation of PTP1B by **1**. Second, addition of $FeSO_4$ (5 μM) inhibits, rather than facilitates, the inactivation process, presumably due to the fact that Fe(II) mediates destruction of the peracid.[29] With the Fenton-type mechanism excluded, it is reasonable to suspect[26–28] that the inactivation of PTP1B by **1** proceeds via the mechanism shown in Scheme 2A.

We find that aromatic peracids **2** and **3** inactivate PTP1B even more effectively than **1** (Fig. 3). For example, **2** and **3**, at 150 nM