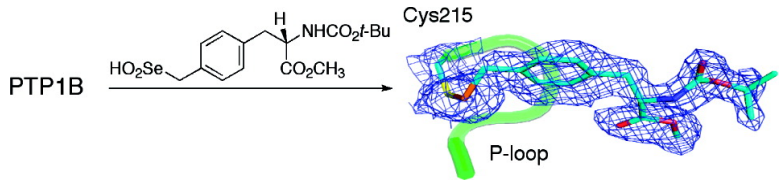
Communication

Seleninate in Place of Phosphate: Irreversible Inhibition of Protein Tyrosine Phosphatases

Mohannad Abdo, Sijiu Liu, Bo Zhou, Chad D. Walls, Li Wu, Spencer Knapp, and Zhong-Yin Zhang

J. Am. Chem. Soc., **2008**, 130 (40), 13196-13197 • DOI: 10.1021/ja804489m • Publication Date (Web): 10 September 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S COMMUNICATIONS

Published on Web 09/10/2008

Seleninate in Place of Phosphate: Irreversible Inhibition of Protein Tyrosine Phosphatases

Mohannad Abdo,[†] Sijiu Liu,[‡] Bo Zhou,[‡] Chad D. Walls,[‡] Li Wu,[‡] Spencer Knapp,*[,†] and Zhong-Yin Zhang*[,‡]

Department of Chemistry and Chemical Biology, Rutgers The State University of New Jersey, Piscataway, New Jersey 08854, and Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202

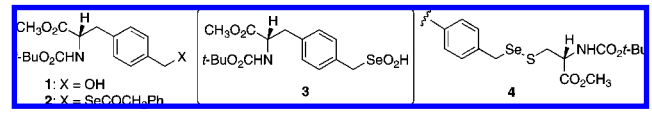
Received June 18, 2008; E-mail: spencer.knapp@rutgers.edu; zyzhang@iupui.edu

Protein tyrosine phosphorylation is a major post-translational mechanism for cellular signaling in response to growth factors, hormones, and cytokines. The extent and duration of tyrosine phosphorylation are tightly regulated by the activities of protein tyrosine kinases and protein tyrosine phosphatases (PTPs). Similar to the kinases, the PTPs constitute a large family of enzymes;[1] malfunction in PTP activity has been associated with human diseases, including cancer, diabetes and obesity, and autoimmune disorders. The PTPs share a conserved active site that recognizes phosphotyrosine (pTyr) and use a common catalytic mechanism that features a highly nucleophilic Cys residue.[2] Among various promising approaches, the development of small molecule modulators of PTP activity could aid in the study of PTP function in complex signaling cascades.

Organoselenium compounds[3] have been found or induced in Nature[4] and have been studied in the laboratory in all four oxidation states: selenol, selenenic, seleninic, and selenonic.[5] Seleninic acids ($RSeO_2H$) are unique in that they can be either protonated or deprotonated[6] at physiological pH and can accept Lewis bases at selenium to form tetracoordinate trigonal bipyramidal adducts (selenuranes).[7] Furthermore, seleninic acids can couple with thiols over a wide pH range to give a redox product, the mixed selenosulfide ($RSeSR'$).[8] The anionic seleninate has been advanced as a bioisostere for carboxylate,[9] inasmuch as it possesses a similar size and charge and can be expected to resist enzymes that process RCO_2^-. $RSeO_2^-$ is also a potential bioisosteric match for the biologically ubiquitous *O*-phosphate group ($ROPO_3^{-2}$),[10] even though the latter is doubly charged. We report that, for a PTP substrate, replacement of the phosphate from tyrosine$-OPO_3^{-2}$ with $-CH_2SeO_2^-$ provides a new inhibitor[11] **3** that reacts effectively and irreversibly with the PTPs in a manner consistent with formation of a covalent selenosulfide adduct.

Seleninic acid **3** was prepared from the homotyrosine derivative[12] **1** by selenocarboxylate Mitsunobu conversion[13] (78% yield) to the selenoester **2** and then reaction of **2** with DMDO[14] in wet acetone solution at 23 °C; **3** was formed (88%) without any overoxidation to the selenonate.[15] The product was purified by silica chromatography and was stable for days stored neat at 23 °C, or in methanol or DMSO solution. The reaction in wet dichloromethane of **3** (1 equiv) with the sulfhydryl of a model Cys derivative gave the selenosulfide **4** in 82% yield, thus demonstrating the feasibility of coupling **3** with the PTP active site Cys residue.

In the PTP catalytic mechanism, the active site Cys sits at the bottom of the pTyr-binding pocket (i.e., the active site) such that its Sγ atom is poised 3 Å from the phosphorus atom of the substrate ready for nucleophilic attack.[2] Incubation of **3** with the *Yersinia*



PTP, YopH, resulted in a time- and concentration-dependent loss of phosphatase activity (Figure 1A). Similar results were obtained with the mammalian enzymes PTP1B as well as the dual specificity phosphatases VHR and VHX (Supporting Information and Figure S1). Inactivation of the PTPs by **3** appeared to be irreversible, as extensive dialysis and/or buffer exchange of the reaction mixture failed to restore enzyme activity. Analysis of the pseudo-first-order rate constant as a function of inhibitor concentration showed that **3**-mediated YopH inactivation displayed saturation kinetics (Figure 1B), yielding values for the binding constant K_I and the inactivation rate constant k_i of 44.5 ± 8.8 μM and 0.243 ± 0.009 min^{-1}, respectively. These results suggest that **3** is an active site-directed affinity agent whose mode of action involves at least two steps: binding to the PTP active site, followed by covalent modification of active site residue(s). In further support of this idea, the active site-directed competitive YopH inhibitors arsenate and *p*-nitrocatechol sulfate were each able to partially protect YopH from **3**-mediated inactivation (Figures S2 and S3).

To directly demonstrate that inhibitor **3** inactivates the PTPs by covalent modification, we analyzed YopH treated with or without **3** using mass spectrometry (Figure S4). The mass of unmodified YopH PTP domain (residues 163−468) measured by an electrospray ion trap mass spectrometer was 33 512 Da, which is in close agreement with the theoretical value (33 513 Da). YopH treated with **3** showed an altered mass of 33 884 Da. This corresponds to a mass difference of 372 Da, consistent with the formation of a mixed selenosulfide covalent adduct between YopH and **3** (the theoretical mass shift is 371 Da). Although a selenosulfide bond is normally readily cleaved by DTT in buffer solution,[16] the selenosulfide linkage in the modified YopH is comparatively stable, inasmuch as mM concentrations of DTT failed to reactivate the inhibitor **3** treated enzyme. Thus, the (PTP)S−SeR′ bond is probably protected from DTT by being buried in the active site. Finally, **3** failed to modify the catalytically inactive mutant YopH/C403S (Figure S5), consistent with formation of the covalent adduct between YopH and **3** at the active site Cys403 explicitly.

To further define the details of PTP inactivation by **3**, we obtained a 2.3 Å crystal structure of PTP1B·**3** complex, as described in the Supporting Information and Table S1. The final model for the PTP1B·**3** complex includes PTP1B residues 2−283 and all non-H atoms in **3**. The overall structure of PTP1B·**3** is similar to the previously determined ligand-free PTP1B structure.[17] The major difference between these two structures is the electron density in

[†] Rutgers.
[‡] Indiana University.

10.1021/ja804489m CCC: $40.75

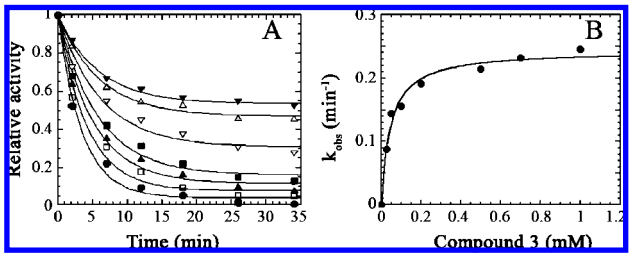


Figure 1. Kinetic analysis of YopH inactivation by **3** at 25 °C and pH 6. (A) Time and concentration dependence of inhibitor **3**-mediated YopH inactivation. (B) Concentration dependence of the pseudo-first-order rate constants k_{obs} for **3**-mediated YopH inactivation.

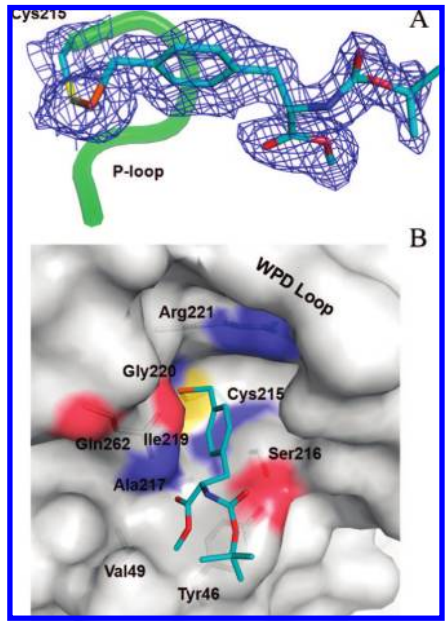


Figure 2. Crystal structure of PTP1B·**3** complex. (A) Electron density for **3** and Cys215 contoured at 1.0 δ level for the F_o-F_c map. The P-loop is depicted as a green cartoon. Active site Cys215 and **3** are shown in stick model. (B) Interactions between PTP1B and **3**. Inhibitor **3** is shown in stick model with atom color: oxygen, red; carbon, cyan; sulfur, yellow; Se, orange.

the PTP active site corresponding to **3**, which is covalently attached to PTP1B via a selenosulfide bond between Cys215 Sγ and the selenium atom in **3** (Figure 2). The presence of electron density for the covalently attached **3** was confirmed by analyzing the $2F_o-F_c$ and F_o-F_c difference Fourier maps with contour levels of 1.0 and 2.5 δ, respectively (Figure 2A). The bond length for the S−Se linkage is 2.2 Å, in good agreement with the theoretical value (2.1 Å).[18] The structural data provide direct evidence that inhibitor **3** specifically inactivates the PTPs by forming a mixed selenosulfide with the active site Cys residue.

In addition to revealing the nature of covalent linkage between PTP1B and **3**, the structure also identifies additional noncovalent interactions between the active site of PTP1B and **3**. As shown in Figure 2B, inhibitor **3** is inserted into the active site pocket that is capped by the P-loop (β8-α5, residues 215−221). At the center of the P-loop lies the catalytic Cys215, which forms a covalent bond with the selenium atom in **3**. The highly flexible WPD loop adopts an open conformation in the PTP1B·**3** structure. The selenium atom makes two polar interactions with the main-chain nitrogens of Gly220 and Arg221. It is also within van der Waals contacts with main-chain carbon atoms of Ile219, Gly220, and Arg221 as well as the side chain of Arg221 (Figure S6). A H-bond exists between the carbonyl oxygen in the methyl ester and the side chain of Gln262. The phenyl ring of **3** is engaged in extensive hydrophobic interactions with the residues lining the active site cavity, including Tyr46, Ser216, Ala217, and Arg221 (Figures 2B and S6). Hydrophobic interactions are also observed between the benzylic methylene next to the selenium and the side chain of Arg221, while the carbonyl carbon and the methyl group in the methyl ester interacts with the side chains of Tyr46 and Val49, respectively. Finally, the *tert*-butyl group in **3** also makes contacts with Tyr46 (Figures 2B and S6). These noncovalent interactions likely contribute to the efficient PTP inactivation by **3**.

The kinetic, mass spectrometry, and X-ray crystallographic data together demonstrate that **3** is an effective pTyr surrogate that can specifically modify the PTP active site Cys residue by forming a stable selenosulfide linkage. It is worthwhile to note that the kinetic parameters K_I and k_i for inhibitor **3**-mediated YopH inactivation (44.5 μM and 0.243 min^{-1}) compare very favorably to those determined for the previously described α-bromobenzyl phosphonate based probe (4.1 mM and 0.11 min^{-1}).[19] These observations highlight the potential for developing seleninate based small molecule probes to modulate PTP activity in signaling and in diseases.

Acknowledgment. Dedicated to Prof. J. Meinwald on the occasion of his 80th birthday. We are thankful to the Prusoff Foundation for partial financial support at Rutgers and to Rohm and Haas Co. for a graduate assistantship to M.A. The work at Indiana University was supported by NIH CA126937.

Supporting Information Available: Experimental details for **2**−**4**, and description of PTP inhibition assays, mass spectrometry, and crystallographic characterization of PTP1B·**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Alonso, A.; Sasin, J.; Bottini, N.; Friedberg, I.; Friedberg, I.; Osterman, A.; Godzik, A.; Hunter, T.; Dixon, J.; Mustelin, T. *Cell* **2004**, *117*, 699–711.
(2) Zhang, Z.-Y. *Prog. Nucleic Acid Res. Mol. Biol.* **2003**, *73*, 171–220.
(3) Klayman, D. L. In *Organic Selenium Compounds*, Klayman, D. L., Günther, W. H. H., Eds.; Wiley Interscience: New York, 1973; pp 67−171.
(4) (a) Block, E.; Birringer, M.; Jiang, W.; Nakahodo, T.; Thompson, H. J.; Toscano, P. J.; Uzar, H.; Zhang, X.; Zhu, Z. *J. Agric. Food Chem.* **2001**, *49*, 458–470. (b) Olson, O. E.; Novacek, E. J.; Whitehead, E. I.; Palmer, I. S. *Phytochem.* **1970**, *9*, 1181–1188.
(5) Paulmier, C. *Selenium Reagents and Intermediates in Organic Synthesis*; Pergamon Press: Oxford, 1986; pp 25−57.
(6) McCullough, J. D.; Gould, E. S. *J. Am. Chem. Soc.* **1949**, *71*, 674–676.
(7) Hargittai, I.; Rossandai, B. In *The Chemistry of Organic Selenium and Tellurium Compounds*; Patai, S., Rappoport, Z., Eds.; John Wiley and Sons: Chichester, 1986; Vol. 1, pp 125−143.
(8) Kice; J. L.; Lee, T. W. S. *J. Am. Chem. Soc.* **1978**, *100*, 5094–5102.
(9) Stuhr-Hansen, N.; Ebert, B.; Krogsgaard-Larsen, P.; Kehler, J. *Org. Lett.* **2000**, *4*, 7–9.
(10) Rye, C. S.; Baell, J. B. *Curr. Med. Chem.* **2005**, *12*, 3127–3141.
(11) Various related homotyrosine *sulfonates* have been studied, but not, to our knowledge, the *sulfinates* or any organoselenium species: (a) Herzner, H.; Kunz, H. *Tetrahedron* **2007**, *63*, 6423–6436. (b) Hill, B.; Ahmed, V.; Bates, D.; Taylor, S. D. *J. Org. Chem.* **2006**, *71*, 8190–8197. (c) Beaulieu, P. L.; Cameron, D. R.; Ferland, J.-M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, R.; Jakes, S.; Kabcenell, A.; Kirrane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. *J. Med. Chem.* **1999**, *42*, 1757–1766.
(12) (a) Morera, E.; Ortar, G.; Varani, A. *Synth. Commun.* **1998**, *28*, 4279–4285. (b) Marseigne, I.; Roques, B. P. *J. Org. Chem.* **1988**, *53*, 3621–3624.
(13) Knapp, S.; Darout, E. *Org. Lett.* **2005**, *7*, 203–206.
(14) Knapp, S.; Darout, E.; Amorelli, B. *J. Org. Chem.* **2006**, *71*, 1380–1389.
(15) DMDO preparation of seleninates from selenoesters: Abdo, M.; Knapp, S. *J. Am. Chem. Soc.* **2008**, *130*, 9234–9235.
(16) Besse, D.; Siedler, F.; Diercks, T.; Kessler, H.; Moroder, L. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 883–885.
(17) Pedersen, A. K.; Peters, G. H.; Moller, K. B.; Iversen, L. F.; Kastrup, J. S. *Acta Crystallogr., Sect. D* **2004**, *60*, 1527–1534.
(18) Hanzelmann, P.; Dobbek, H.; Gremer, L.; Huber, R.; Meyer, O. *J. Mol. Biol.* **2000**, *301*, 1221–1235.
(19) Kumar, S.; Zhou, B.; Liang, F.; Wang, W.-Q.; Huang, Z.; Zhang, Z.-Y. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7943–7948.

JA804489M