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COMMUNICATIONS

Published on Web 07/19/2005

A Chemical Model for Redox Regulation of Protein Tyrosine Phosphatase 1B (PTP1B) Activity

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The functional properties of many proteins involved in cellular signal transduction are modulated by the addition and removal of phosphoryl groups at selected serine, threonine, and tyrosine residues.[1] The phosphorylation status of target amino acid residues in relevant proteins is controlled by the coordinated action of protein kinases[2] that catalyze the addition of phosphoryl groups and protein phosphatases[3] that catalyze the removal of these groups. The cellular activity of various kinases and phosphatases must be tightly regulated[1−3] for normal cell function, and it is important to seek an understanding of the molecular processes governing the activity of these enzymes.

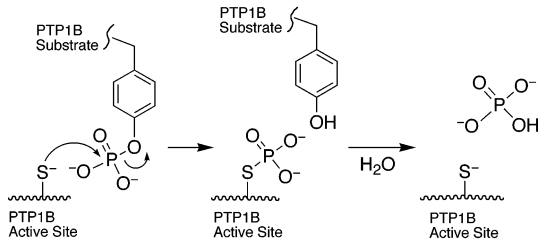
Growing evidence indicates that endogenously produced hydrogen peroxide acts as a cellular signaling molecule that (among other things) can regulate the activity of some protein phosphatases.[4] For example, exposure of cells to agents such as insulin, growth factors, and cytokines elicits a burst of hydrogen peroxide that inactivates selected phosphatases and leads to an increase in phosphorylation levels of relevant substrate proteins.[5] Hydrogen peroxide-mediated inactivation of phosphatases is typically thought to involve oxidation of a catalytic thiol residue as shown in Scheme 1.[5d,6] This process is reversible, and when levels of hydrogen peroxide decline, reactions with the cellular thiol glutathione convert the inactivated protein back to its catalytically active form (this process may be catalyzed by disulfide reductases such as thioredoxin or glutaredoxin).[5e,6f]

Protein tyrosine phosphatase 1B (PTP1B) plays a central role in insulin signaling.[7] A key feature of this signal transduction pathway involves the transient inactivation of PTP1B by an insulin-stimulated burst of hydrogen peroxide.[5a] Recent X-ray crystallographic studies revealed an unexpected chemical transformation underlying the redox regulation of PTP1B in which oxidative inactivation of the enzyme yields an intrastrand protein cross-link between the catalytic cysteine residue and a neighboring amide nitrogen (Scheme 2).[8,9] Several possible mechanisms were offered[8,9] to explain the formation of this protein-derived 3-isothiazolidinone heterocycle, with the most direct route involving peroxide-mediated oxidation of the active-site thiol residue to a sulfenic acid, followed by attack of the neighboring amide nitrogen (Scheme 2). This transformation is unprecedented in the literature of both organic chemistry and protein chemistry and is striking because nitrogen atoms of amide groups are generally considered poor nucleophiles.[10]

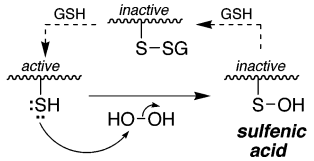
Here, we set out to develop a small organic model system[11] that could be used to characterize the unusual chemical reactions involved in the redox regulation of PTP1B. A central goal of this initial study was to determine whether the sulfenic acid intermediate is, in fact, competent to generate the 3-isothiazolidinone heterocycle as shown in Scheme 2. For this purpose, we prepared compounds **1a** and **1b** in three steps from thiosalicylic acid. Importantly, we determined that the thiol form of this model system (e.g. **5a**, Scheme 3) has a pK_a of 5.7, similar to that measured for the active-site thiol in PTP1B (pK_a = 5.6).[12] Sulfenic acids typically are too unstable to be isolated;[13] thus, we employed β-sulfinyl propionic acid ester groups as a means[14,15] for in situ generation of the desired

Scheme 1

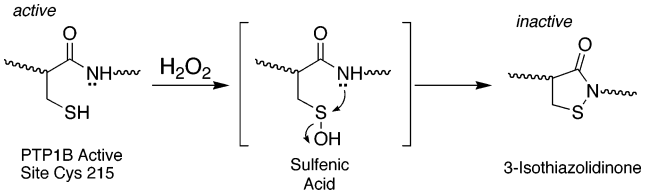
Reaction Catalyzed by Protein Tyrosine Phosphatases



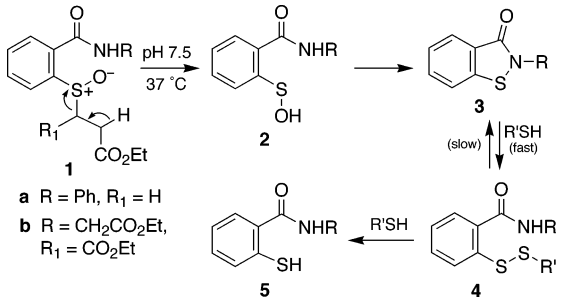
"Typical" Mechanism for Redox Regulation of Cys-Dependent Protein Tyrosine Phosphatases



Scheme 2



Scheme 3

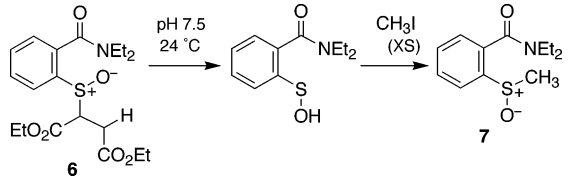


sulfenic acid intermediate under physiologically relevant conditions. Finally, the ortho-substituted benzene scaffold of **1** provides a good model for the enforced proximity of the amide and cysteine thiol groups at the active site of PTP1B.[8,9]

We find that incubation of **1b** in aqueous buffered solution (250 mM sodium phosphate, pH 7.5, containing 30% MeCN at 37 °C) affords a 92% yield of the 1,2-benzisothiazolin-3(2*H*)-one **3b** (Scheme 3). The reaction is rather robust and does not depend strongly upon the nature of the solvent or the substituent on nitrogen. For example, the phenyl-substituted amide **1a** gave an 88% yield of **3a** in the sodium phosphate buffer system described above, and **1b** provides a 60% yield of **3b** when the reaction is conducted in

10.1021/ja052599e CCC: $30.25

Scheme 4



dichloromethane. Consistent with our expectation[14,15] that the β-sulfinyl propionic acid ester group would decompose to produce the desired sulfenic acids under these reaction conditions, we find that the analogue **6**, in which cyclization to the benzisothiazolinone ring system is blocked by dialkyl substitution on the amide nitrogen, yields the characteristic[13] product (**7**) resulting from alkylation of the sulfenic acid intermediate when incubated in the presence of excess methyl iodide (Scheme 4).[16] In the absence of methyl iodide, this compound affords the expected[13] products arising from sulfenic acid dimerization (see Supporting Information). In direct analogy to the oxidative inactivation of PTP1B shown in Scheme 2, we find that treatment of the thiol **5a** with H_2O_2 produces a good yield of **3a** (see Supporting Information).

Taken together, the results indicate first that the compounds **1** decompose to yield the sulfenic acid intermediates **2** and second that the sulfenic acids (**2**) undergo efficient conversion to the 3-isothiazolidinone products **3**.[17] In contrast, the mixed disulfide **4b** (Scheme 3, R′ = CH_2CH_2OH) does *not* undergo rapid cyclization to **3b** either in organic solvent ($CDCl_3$) or our standard sodium phosphate buffer system.[18] Overall, our findings indicate that the oxidative transformation of PTP1B to its inactive 3-isothiazolidinone form can proceed directly via oxidation of the active-site thiol to a sulfenic acid intermediate (as shown in Scheme 2). This argues against the need to invoke alternative mechanisms[9] involving further conversion of the sulfenic acid to a sulfenyl peroxide or a mixed disulfide.

In addition to modeling the chemistry underlying oxidative inactivation of PTP1B, the small organic system reported here also mimics the thiol-mediated reduction of the inactive isothiazolidinone form of the enzyme back to its catalytically active thiol form.[8,9] Specifically, **3b** is rapidly and completely converted (<1 min) to the aromatic thiol **5b** (Scheme 3) upon treatment with excess thiol (10 μM **3b**, 10 equiv of 2-mercaptoethanol, in 50 mM pH 7.0 phosphate buffer containing 30% acetonitrile).

In summary, we have developed a small organic molecule that serves as an effective model for the redox-sensing assembly of functional groups found at the active site of the enzyme PTP1B. Importantly, results obtained with this model system show that the sulfenic acid residue possesses sufficient electrophilicity to drive the cyclization reaction with a neighboring amide group, thus generating a 3-isothiazolidinone heterocycle analogous to that recently characterized at the active site of oxidatively inactivated PTP1B.[8,9] Protein sulfenic acids are common intermediates generated during the oxidation of cysteine thiol residues in cells.[19] This fact, along with the remarkably facile nature of the sulfenic acid chemistry reported here, suggests that the reversible formation of a protein-derived 3-isothiazolidinone residue first seen in the context of PTP1B represents a potentially *general* mechanism for redox "switching" of protein function. Thus, this chemistry could have broad relevance to both redox-regulated signal transduction and the toxic effects of oxidative stress.

Acknowledgment. We thank Professors Peter Tipton, Richard Loeppky, and Christina Wells for critical review of the manuscript and are grateful to the NIH (CA 83925) for partial support of this work.

Supporting Information Available: Experimental procedures and spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) The formation of **3** from **1**, in principle, could occur via a process involving dimerization of the sulfenic acid **2** to the corresponding thiosulfinate (RS(O)SR), followed by conversion of the thiosulfinate to **3**. However, this possibility is rendered unlikely based upon our observation (see Supporting Information) that the yield of the cyclization product **3** (Scheme 3) is not diminished when the reaction is conducted in the presence of methyl iodide concentrations that completely *prevent* dimerization of a sulfenic acid intermediate as shown in Scheme 4.
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JA052599E