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Selective Inactivation of Protein Tyrosine Phosphatase PTP1B by Sulfone Analogue of Naphthoquinone

Seung Wook Ham,* Junguk Park, Soo-Jin Lee, and Ji Sang Yoo

Department of Chemistry, Chung-Ang University, Seoul 156-756, Korea

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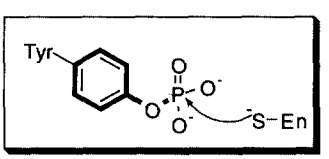
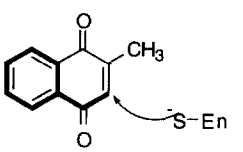
Abstract: Protein tyrosine phosphatase inactivators are of interest as research tools and as therapeutic agents. In this study, the effect of sulfone analogue of naphthoquinone on the activities of PTP1B and other PTPs was examined. The results indicated that this compound selectively and irreversibly inactivated the PTP1B with the dissociation constant K_i of 3.5 μM and the inactivation rate constant k_{inact} of 2.2 x 10^{-2} sec^{-1}.

Keywords: Antitumour compds; Enzyme inhibitors; kinetics; phosphorylaton.

Phosphorylation of proteins on tyrosyl residues is a central element of many cellular processes such as cell growth, proliferation, and differentiation [1]. The level of tyrosyl group phosphorylation depends on the relative activity of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Since the removal of the phosphoryl group from phosphotyrosine residue(s) in protein substrates is associated with a number of diseases, including several cancers and diabetes [2], PTP inhibitors may not only serve as valuable probes for studying cellular signal transduction, but may ultimately constitute a family of therapeutic agents.

Recent work from our laboratory has shown that protein phosphatase was inactivated by





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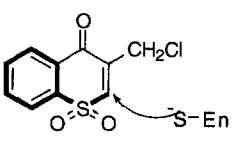

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menadione (2-methyl-1,4-naphthoquinone) **1** and that the loss of enzyme activity was due to the modification of the active site [3]. In the course of searching for new enzyme inactivators, we found in the literature that sulfone analogues of naphthoquinone **2** possess antitumor activity [4]. Therefore, we tested the ability of compound **2** to inactivate the PTPs, which were either purchased or purified according to described method. Unexpectedly, while the compound **2** (up to 40 μM) showed no inactivation of the protein phosphatases cdc25A, cdc25B, cdc25C, LAR, and *Yersinia* PTP, **2** resulted in selective inactivation of the PTP1B (residues 1-321) [5] (Figure 1).

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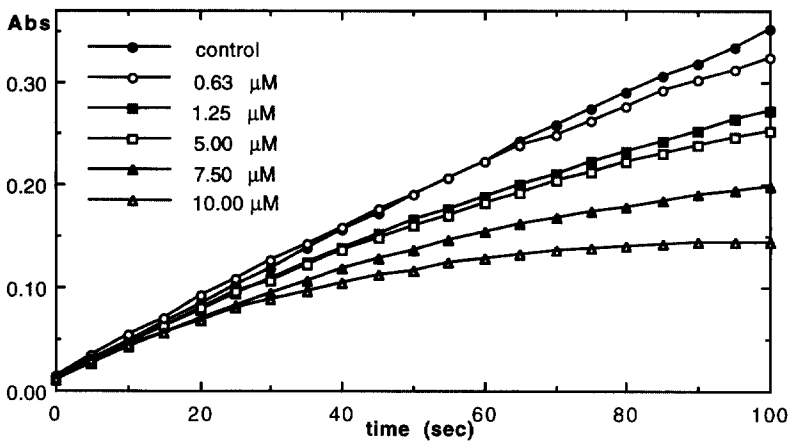

Figure 1. Time course of the reaction of PTP1B in the absence of or presence of the compound **2**. PTP1B (1 μM) was incubated at 37°C with chromogenic substrate, 40 mM *p*-nitrophenyl phosphate in 40 mM Bis-Tris-HCl (pH 7.0), 50 mM NaCl, and 1 mM EDTA [5]. The reaction was continuously monitored at 410 nm by a JASCO UV/Vis spectrophotometer equipped with a JASCO peltier type thermostatic cell holder.

When the PTP1B treated by the compound **2** was dialyzed for 2 days at 4°C against the buffer, the enzyme was not reactivated, indicating an irreversible inactivation process. Moreover, inactivation of PTP1B was protected by the competitive inhibitor, arsenate, suggesting that inactivation occurred at the active site. The efficiency of the inactivator was also evaluated by determining the K_i and k_{inact} using the method of Kitz and Wilson [6]. The values found for the dissociation constant K_i and the inactivation rate constant k_{inact} were 3.5 ± 0.5 μM and 2.2 ± 0.2 x 10^{-2} sec^{-1}, respectively. These values compare most favorably with those for mechanism-based inhibitor of PTP. To the best our knowledge, this is the first report of a selective, irreversible PTP1B inactivator [7]. We are examining the binding interaction of **2** with the catalytic site of PTP1B by solving the X-ray structure of the inactivator enzyme complex.

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