Oxidizing Compounds as Inhibitors of Protein Tyrosine Phosphatases Activity

Alicja Kuban-Jankowska, Magdalena Gorska-Ponikowska, and Pawel Niedzialkowski

***Abstract*—**The results of several studies suggest that the production of reactive oxygen species associated with oxidative stress, may cause inactivation of the protein tyrosine phosphatases (PTPs), and reversible oxidation catalytic cysteine was found to be a major mechanism for regulating their activity. The oxidizing compounds may therefore be considered as protein tyrosine phosphatases inhibitors. In the present paper we investigated the effect of reactive oxygen species such as hydrogen peroxide and activated by him fatty acids on enzymatic activity of protein tyrosine phosphatase PTP1B. Here we presented the IC_{50} values of hydrogen peroxide, peroctanoic acid and ATA against PTP1B phosphatase, showing that the highest level of inactivation were caused by peroctanoic acid, a product of octanoic acid activated by hydrogen peroxide. However this compound is highly reactive and it can be difficult to consider it as a drug. Taking to account IC_{50} values of tested compounds we suggest that ATA can be potent inhibitor of PTP1B. The spectroscopic studies also confirmed that inhibitory action of ATA is associated with generation of hydrogen peroxide, which allow to conclude that ATA inactivates PTP1B by oxidation.

***Keywords*—**Catalytic cysteine residue, PTP1B phosphatase, reversible oxidation.

I. Introduction

PTPs regulate the cellular level of tyrosine phosphorylation under normal and pathological conditions, having both positive and negative effects on cellular signal transduction [1]. Any disfunction of their activity may lead to the development of numerous human diseases. Disregulations of PTPs action contribute to the development of cancer, type II diabetes, or cardiovascular and neurological diseases [2]. The role of protein tyrosine phosphatases in the formation and development of tumors was presented during the implementation of a number of scientific research. The participation of PTPs in development of glioma, colorectal cancer, lung cancer, breast cancer, and multiple myeloma has been proven. Phosphatases PTP1B and Shp2 are particularly important target in the treatment of breast cancer [3, 4]. A current research on pathophysiological roles of PTPs has focused on transgenic or knockout mice studies, best highlighted by the discovery of the role of PTP1B in type II diabetes and obesity. The inhibition of the signaling function of PTP1B may be exploited for therapeutic intervention in diabetes and obesity [5]. It has been found that mice lacking PTP1B phosphatase are healthy and have enhanced sensitivity to insulin. Moreover, mice with PTP1B deletion are lean and protected from diet-induced obesity [6].

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II. Materials and Methods

A. Inhibitory activity analysis

We analyzed the enzymatic activity of recombinant PTP1B phosphatase after treatment with several concentrations of selected inhibitors and calculated IC_{50} values of hydrogen peroxide, peroctanoic acid and aurintricarboxylic acid. The enzymatic activity of PTP1B was measured using 1mM chromogenic substrate *para*-nitrophenyl phosphate (*p*NPP) in 50mM Tris buffer, pH 7.4, at 37ºC. The increase in absorbance (due to *para*-nitrophenol formation) is linearly proportional to enzymatic activity concentration (with excessive substrate, i.e. zero-order kinetics) and was assessed at 405 nm on a microplate reader Jupiter (Biogenet) using DigiRead Communication Software (Asys Hitech GmbH).

B. Spectroscopic studies

We prepared also spectroscopic studies of aurintricarboxylic acid measuring fluorescence emission spectra and fluorescence excitation spectra of ATA and O_2.

III. Results

A. Inhibitory Activity of Selected Inhibitors Against PTP1B Phosphatase

We compared inhibitory properties of selected oxidizing compounds such as, hydrogen peroxide, peroctanoic acid and aurintricarboxylic acid against enzymatic activity of recombinant PTP1B phosphatase. The calculated IC_{50} values are presented in a Table I.

B. Fluorescence Emission Spectra

We performed spectroscopic studies of potent inhibitor of PTPs aurintricarboxylic acid measuring fluorescence emission

spectra and fluorescence excitation spectra of ATA and O_2 (Fig. 1).

According to the Kasha's rule, fluorescence takes place from the lowest excited state. That implies independence of the fluorescence spectra on the excitation wavelength. This rule can't be corrupted in case of molecules, which fragments can rotate or vibrate. So, ATA should have the same pink fluorescence (max 600 nm), when excited either at 550, 475, 315 or 280 nm. This very low intensive fluorescence (fluorescence quantum yield << 0. 01%) is observed in case of excitation at 550 and 475 nm. However, excitation at 280 and 315 nm gives rise to a more intensive blue fluorescence (max 406 nm). Most probably, this one originates from different species.

C. Fluorescence Excitation Spectra

In a simple case, when the only one type of species absorb and emit light, excitation fluorescence spectra is identical to the absorption one. Any difference between excitation and absorption spectra indicates existence of more than one type of fluorescent species in solution. Importantly, the intensity of the fluorescence excitation spectra depends on the fluorescent properties of compound – highly fluorescent compound has intensive excitation spectra, whilst non-fluorescent can't be registered by this technique.

In the current case the excitation fluorescence spectra of the species with the blue fluorescence (max at 406 nm) consists at least of two bands at 290-300 and 340 nm, which intensity differs depending on the O_2 content. None of those bands correspond to the band observed in the absorption spectra at the same region (315 nm) (Fig. 2).

In summary, most probably, that the ATA fluorescence is not influenced by complexation with O_2. The sample contains a blue-fluorescent impurity, which is registered in the blue region because of much higher fluorescence quantum yield, compared to ATA. As we deal with a mixture of compounds, fluorescence quantum yield can't be determined. Since ATA is usually synthesized from salicylic acid, which fluorises at 405-410 nm, its traces can be a reason of the observed fluorescence behavior.

IV. Discussion

It is believed that the reversible oxidation of catalytic cysteine can be a universal mechanism for regulating the activity of protein tyrosine phosphatases. The role of protein tyrosine phosphatases in the formation of tumors can be partly related to increased production of reactive oxygen species (ROS) in tumor cells when compared to normal cells that can lead to impaired regulation of PTP oxidation. Furthermore, overexpression of the protein Nox1 producing reactive oxygen species can cause cellular transformation and tumorigenesis, confirming the participation of ROS in cancer development. Hydrogen peroxide can inhibit protein tyrosine phosphatases by oxidation catalytic cysteine residue [7]. In the present paper we investigated the effect of reactive oxygen species such as hydrogen peroxide and activated by him octanoic acid, as well as ATA, generator of hydrogen peroxide, on enzymatic activity of phosphatase PTP1B.

Oxidative stress due to reactive oxygen species formation may induce inactivation of PTPs. Inactivation after oxidation was suggested as a general mechanism for regulation of PTPs [8].

The unique biochemical and structural characteristics of the catalytic cysteine, highly sensitive for oxidation, in the active site of PTPs engendered the hypothesis that these enzymes might be direct targets of ROS. Many PTPs have been shown to be oxidized transiently in response to various cellular stimuli. ROS, such as hydrogen peroxide, function as second messengers in response to extracellular stimuli and can regulate tyrosine phosphorylation signaling pathways [9].

The essential cysteine residue in a catalytic site of PTPs exists as a thiolate anion at neutral pH and due to their microenvironment has an extremely low pK_a. These properties allow cysteine residue to function as a nucleophile in catalytic action, but cause it is highly vulnerable to oxidation [10]. Depending upon the extent of oxidation, the catalytic cysteine residue can be converted to either sulfenic (SOH), sufinic (SO_2H) or sulfonic (SO_3H) acid residues (Fig. 3) [11].

Hydrogen peroxide may relatively easily cross the cell membrane in response to insulin or epidermal growth factor, and control the cellular activity of PTPs inside the cell [12]. Hydrogen peroxide may oxidize catalytic cysteine residue to sulfenic acid, which can be reversibly reduced to cysteine by various cellular reducing agents [13].

Very powerful oxidants that may lead to oxidation of proteins are peroxidized acids. It has been found that peroxidized arachidonic acid may induce oxidation of PTPs [14]. Another study has shown that peracetic acid is a potent oxidative inhibitor of PTP1B phosphatase [15]. Our previous studies has confirmed that peracids are potent inhibitors of PTPs [16].

The strongest PTPs inhibitor characterized is aurintricarboxylic acid (ATA), which inhibit the phosphatase activity in nanomolar concentrations. It was suggested that ATA is able to generate hydrogen peroxide. Here we decided to focus on oxidizing properties of this compound.

V. Conclusion

Protein tyrosine phosphatases serve as key mediators of cell signaling and their activity is strictly related to reactive oxygen species (ROS) level and action. The reversible oxidation is the main mechanism of control of PTPs activity [17]. PTPs are essential enzymes that regulate the tyrosine phosphorylation process which control cell adhesion and migration. Inhibitors of PTP1B can be utilized in a treatment of many disorders associated with its disfunction. Here we analyzed and compared the inhibitory properties against PTP1B of selected PTPs inhibitors, such as hydrogen peroxide, peroctanoic acid and aurintricarboxylic acid (ATA), which decrease the

phosphatase activity by oxidation of catalytic cysteine in the active site. We performed as well the spectroscopic studies of potent inhibitor of PTPs, aurintricarboxylic acid. We found that hydrogen peroxide inhibit PTP1B phosphatase with IC_{50} value equal to 200 nM. Peroctanoic acid, which is a product of octanoic acid activated by hydrogen peroxide, is able to inactivate PTP1B with IC_{50} value equal to 50 nM. It is the strongest inhibitor tested but also the most reactive one. Less reactive and still potent inhibitor is ATA with IC_{50} value equal to 100 nM. The spectroscopic studies also confirmed that inhibitory action of ATA is associated with generation of hydrogen peroxide. We suggest that aurintricarboxylic acid can be considered as potent inhibitor of PTP1B phosphatase and in a future may lead to drug design for PTP1B involved human diseases.

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TABLE I. The estimated IC_{50} value of selected inhibitors for PTP1B

Compounds	Calculated estimated IC_{50} values for PTP1B phosphatase
Hydrogen Peroxide	200 nM
Peroctanoic Acid	50 nM
Aurintricarboxylic Acid	100 nM

Fig. 1. The fluorescence emission spectra of ATA and ATA+O_2.

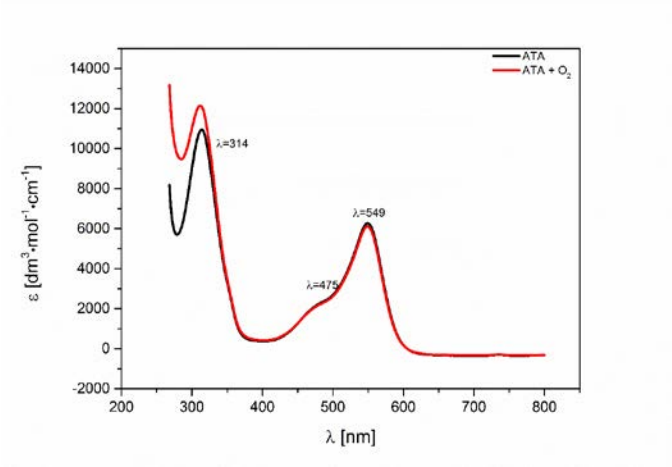
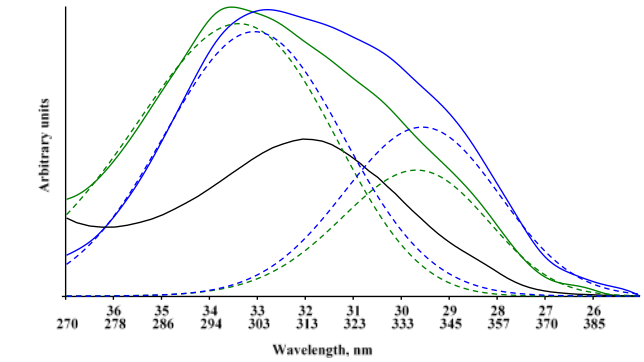


Fig. 2. The fluorescence excitation spectra of ATA.



Black solid line - absorption spectra of the ATA solution in DMSO under Ar.
Green solid line - excitation fluorescence spectra at 450 nm of the same solution under Ar.
Blue solid line - excitation fluorescence spectra at 450 nm of the same solution under O_2.
Dashed lines represent single bands in the excitation fluorescence spectra of solutions under Ar (green) and under O_2 (blue) obtained by deconvolution.

Fig. 3. The reversible and irreversible oxidation of PTPs catalytic cysteine residue to sulfenic, sulfinic or sulfonic acid.