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Mechanisms underlying the inhibitory effects of arsenic compounds on protein tyrosine phosphatase (PTP)

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ABSTRACT

Arsenic binding to biomolecules is considered one of the major toxic mechanisms, which may also be related to the carcinogenic risks of arsenic in humans. At the same time, arsenic is also known to activate the phosphorylation-dependent signaling pathways including the epidermal growth factor receptor, the mitogen-activated protein kinase and insulin/insulin-like growth factor-1 pathways. These signaling pathways originate at the level of receptor tyrosine kinases whose phosphorylation status is regulated by opposing protein tyrosine phosphatase (PTP) activity. Reversible tyrosine phosphorylation, which is governed by the balanced action of protein tyrosine kinases and phosphatases, regulates important signaling pathways that are involved in the control of cell proliferation, adhesion and migration. In the present study, we have focused on the interaction of cellular PTPs with toxic trivalent arsenite (iAs^{III}) and its intermediate metabolites such as monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) in vitro, and then determined the arsenic binding site in PTP by the use of recombinant PTPs (e.g., PTP1B and CD45). Interestingly, the activities of PTP1B (cytoplasm-form) or CD45 (receptor-linked form) were observed to be strongly inhibited by both methylated metabolites (i.e., MMA^{III} and DMA^{III}) but not by iAs^{III}. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has clearly confirmed that the organic intermediate, DMA^{III} directly bound to the active site cysteine residue of PTP1B (e.g., Cys215), resulting in inhibition of enzyme activity. These results suggest that arsenic exposure may disturb the cellular signaling pathways through PTP inactivation.

Introduction

Arsenic, a well-known carcinogen is widely present in drinking water and has been recognized for affecting a large number of populations by its toxic effects via various mechanisms. Conversely it is being widely used in the treatment of acute promyelocytic leukemia (APL) as well as other types of tumors (Ahn et al., 2010; Douer and Tallman, 2005). However, the mechanisms underlying arsenic-induced diseases or anti-cancers effects are not precisely understood, however the binding of arsenicals to biomolecules is considered to be one of the major toxic mechanisms (NRC, 1999, 2001). Moreover, the emerging investigations are striving to their best to suggest the probable and appropriate mechanism of arsenic biotransformation into its reactive metabolites which in future may assist in elucidating the effects of arsenic on health (Rehman and Naranmandura, 2012).

Protein tyrosine phosphorylation is known for its critical role in signal transduction pathways at molecular level which in turn regulates many cellular processes including propagation (Neel and Tonks, 1997), survival, differentiation and apoptosis (Zhang, 2002). Protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) are known to control the level of protein tyrosine phosphorylation (Hunter, 2000). Many diseases like cancers and diabetes have been reported to be associated with irregular tyrosine phosphorylation (Ullrich and Schlessinger, 1990). PTPs, the dephosphorylation enzyme interferes with the signal transduction regulated by receptor tyrosine kinases. Interestingly, PTPs have been recognized for their dual affects on cancer-related signal pathways either exhibiting inhibitory or stimulatory effects, thereby, any disturbance in the functioning of PTP may provoke events of carcinogenesis (Ostman and Böhmer, 2001). These PTPs may be receptor-form like

Abbreviations: iAs^{III}, arsenite; iAs^{V}, arsenate; DMA^{V}, dimethylarsinic acid; DMA^{III}, dimethylarsinous acid; ROS, reactive oxygen species; ICP MS, inductively coupled argon plasma mass spectrometry; PTP, protein tyrosine phosphatase.

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CD45 or cytoplasmic-form like PTP1B, however, cysteine will be found as a common residue in all forms of PTPs which is essential for enzyme activity (Barford et al., 1995). Thus, PTPs may be represented as enzymes composing catalytic domain with active site cysteine residues.

Reversible oxidation of this catalytic active site cysteine of PTP has been widely considered as one of an imperative mechanism for inhibiting the PTP activity. Recent investigation has exposed the susceptibility of catalytic cysteine residue of PTPs towards oxidation which might lead to inactivation of PTPs resulting in increased cellular tyrosine phosphorylation (Lee et al., 1998; Meng et al., 2006). Moreover, (Li and Dixon, 2000) an enhanced perception of the functioning and regulation of PTPs and PTKs might serve as an essential base for understanding the mechanisms of carcinogenesis and development of anticancer drugs as the contrasting reactions of PTPs and PTKs are known to regulate the reversible tyrosine phosphorylation states.

Recently various studies have focused on the interaction between different metalloids and the functioning of PTP to explicate the possible role of these metals on various aspects of health (Andersen et al., 2001). Copper has been identified as PTP inhibitor and has shown to possess high affinity for the active thiolated site of PTPs, thereby it might be considered as a factor that can increase the chances for cytotoxicity (Wang et al., 2010). However, up till date the reactivity of PTP with arsenicals has not been clearly illustrated.

In the present study, we focused on the interaction between cellular PTP1B with the toxic arsenicals including inorganic arsenic (iAs^{III}) and its in vivo organic metabolites, monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}). Additionally, we have utilized recombinant PTP1B for the purpose of determining the binding sites of PTP along with the analysis of PTP interactions with arsenicals.

Material and methods

Reagents

All reagents were of analytical grade. Milli-Q water (Millipore) was used throughout. Trizma® HCl and Trizma® Base were purchased from Sigma (St. Louis, MO, USA). Nitric acid, hydrogen chloride, ammonium acetate, acetic acid, 28% ammonia solution, sodium arsenite (iAs^{III}) and dimethylarsinic acid [$(CH_3)_2AsO(OH)$] (DMA^{V}) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Monomethylarsonic acid (MMA^{V}) was obtained from Tri Chemicals (Yamanashi, Japan). Human protein tyrosine phosphatase 1B human (PTP1B) and human CD45 PTPase were purchased from BIOMOL (Plymouth Meeting, PA). Trypsine, superoxide dismutase (SOD) and catalase were purchased from Sigma-Aldrich (St. Louis, MO). 4-Nitrophenylphosphate was purchased from Tokyo Chemical. Industry Co., Ltd. (Tokyo, Japan). The arsenic standard solution (1000 μg/mL) for ICP MS was purchased from SPEX CentiPrep (Metuchen, NJ, USA). Stock solutions of all arsenic compounds (10 mmol/L) were prepared from the respective standard compounds. All stock solutions were stored in the dark at 4 °C. Diluted standard solutions for analysis were prepared fresh daily.

Preparation of trivalent monomethylarsonous acid (MMA^{III}) and d dimethylarsinous acid (DMA^{III})

MMA^{III} and DMA^{III} were prepared by reducing MMA^{V} and DMA^{V}, respectively, with 5 molar equivalents of L-cysteine in distilled water at 90 °C for 1 h. The trivalent forms were confirmed by comparison of the respective retention times on a GS 220 gel filtration column by HPLC–ICP MS with those prepared from their iodide forms in distilled water under nitrogen atmosphere (Naranmandura et al., 2011, 2012). Purity of MMA^{III} (98%, with 2% of MMA^{V}) and DMA^{III} (95%, and with 5% of DMA^{V}) was confirmed by HPLC–ICP MS, and then used.

HPLC–ICP-MS analysis

The HPLC system consisted of a liquid chromatograph solvent delivery PU-610 pump and a DG 660B-2 degasser (GL Sciences Co., Tokyo). A polymer-based gel filtration column (Shodex Asahipak GS-220 HQ, 300 mm × 7.6 mm i.d., Showa Denko, Tokyo) with an exclusion limit of 3000 Da was used to separate protein-unbound arsenic species from protein-bound arsenicals. A 60-μL aliquot of a sample solution was applied to the column, and then the column was eluted with 50 mM ammonium acetate buffer (pH 6.5 at 25 °C) at a flow rate of 0.6 mL/min. Arsenic in the eluate was monitored with an Agilent 7500ce ICP-MS (Agilent Technologies,Tokyo, Japan) equipped with an octopole reaction system (ORS) with a He flow of 3.0 mL per min to prevent molecular interference by $^{40}Ar^{35}Cl^{+}$ (signal at m/z 75) (Naranmandura and Suzuki, 2008).

Culture of HePG2 cells

HepG2 cells were obtained from the Shanghai Cell Bank of Chinese Academy of Sciences. Cells were seeded at a density of 1.0×10^6 in a 10 cm dish, and were maintained in low glucose Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 mg/mL of streptomycin, at 37 °C under a 5% CO_2 atmosphere. After twenty-four hours post-seeding, cultures were washed twice with phosphate-buffered saline (PBS), and then fresh medium was added, the cells were then treated with iAs^{III}, MMA^{III} and DMA^{III} for 3 h in a dose-dependent manner.

Determination of PTP activity in cell lysates. PTP activity in cell lysates prepared from arsenic-exposed HepG2 cells (i.e., iAs^{III}, MMA^{III} and DMA^{III}) was determined by PTP activity assay. Briefly, cell lysate (80 μg) was incubated with 2 mM pNPP (specific subtract) in 100 mM HEPES buffer (pH 7.4). The reaction was allowed to proceed for 30 min at 37 °C and stopped by addition of 1 M NaOH. The pNP formed in reaction mixtures was determined at 420 nm using a Beckman Coulter DTX-880 Multimode Detector.

Determination of recombinant PTP1B activity

Recombinant proteins PTP1B or CD45 (1 μg/mL) was pre-incubated in 100 mM HEPES buffer (pH 7.4) at 37 °C for 3 min and then exposed to 0, 1, 5, 10 and 20 μM of different arsenic species (i.e., iAs^{III}, MMA^{III} and DMA^{III}) for 60 min. After the incubation, 4-nitrophenyl phosphate (as substrate) was added into the reaction solutions at 1 mM, and then determined the dephosphorylation of 4-nitrophenyl phosphate at 420 nm using a Beckman Coulter DTX-880 Multimode Detector.

Identification of the arsenic-binding fragmentations by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)

Recombinant proteins PTP1B was incubated with trivalent DMA^{III} for 30 min and then digested with trypsin at 37 °C for 12 h. After digestion, the samples were desalted and then concentrated to obtain peptides using a ZipTip C18 pipet tip (Millipore) (Naranmandura and Suzuki, 2008). Briefly, freshly prepared 1,2-dimethoxy-4-3hydroxycinnamic acid (Sigma Aldrich) was used as a matrix solution at a concentration of 10 mg/mL in 50% acetonitrile containing 1% trifluoroacetic acid. An approximately 1.5 μL portion of the desalted and concentrated sample/matrix mixture in the ZipTip C18 pipet tip was directly spotted onto the MALDI target and dried in air. The MALDI-MS measurements were performed with an AXIMA CFRplus time-of-flight mass spectrometer (Shimadzu/Kratos, Kyoto) equipped with a pulsed N2 laser at λ 337 nm, with a pulse width of 3 ns and a frequency of 10 Hz. MALDI mass spectra in the range of m/z 1–5000 were obtained in the positive reflection mode by averaging 200 individual laser shots for 450 μm in the raster scan mode. Mass calibration was carried out according to the