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Formation and characterization of glutathione adducts derived from polybrominated diphenyl ethers

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HIGHLIGHTS

- Twenty PBDE-GSH adducts were synthesized, separated and analyzed by LC-MS.
- Formation mechanism of the PBDE-GSH adducts was proposed and discussed.
- MS/MS fragmentation pathways of the PBDE-GSH adducts were proposed.
- The stability of PBDE-GSH adducts were measured and discussed.
- Formation of GSH-PBDEs adducts may provide valuable information for toxicology study on PBDEs and OH-PBDEs.

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ABSTRACT

The reactions of glutathione (GSH) with polybrominated diphenyl ethers (PBDEs) quinones with different degrees of bromination on the PBDEs ring were studied. Liquid chromatography coupled with mass spectrometric (LC-MS) analysis showed that four types of adducts were formed from the reaction of each PBDEs quinone (PBDE-Q) with GSH. The proposed reaction pathway was confirmed using ion trap-MS/MS technique. Our results demonstrate that adduct-1 was formed following the Michael Addition, a reaction of the sulfhydryl group from GSH with electron deficient carbon from PBDEs-Q ring. Compared with other carbons on the quinone ring, carbon in position 6 has a smaller steric hindrance, thus the addition reaction is likely to occur in that position. Hydrated quinone-GSH adduct-1 is easily oxidized to generate an adduct-2 in an aqueous solution. Substitution reaction from bromine atom on adduct-2 quinone ring with sulfhydryl group from GSH further generates adduct-3 that is unstable and can be readily hydrolyzed to adduct-4 in aqueous solution. Both adduct-1 and adduct-4 were unstable as well, immediately oxidized to adduct-2 and adduct-3 in the air, respectively. The results reveal that brominated quinones undergo a rapid non-enzymatic debromination upon reaction with GSH, and open a new view for our understanding on mechanism of metabolism and the toxicity of this class of compounds.

1. Introduction

As an important class of brominated flame retardants, PBDEs have been widely used in various industrial and consumer goods (Wu et al. (2007); Schecter et al. (2010); Herbstman et al. (2010)). PBDEs have provided people with security as flame retardants, but brought threat to the environment and human health (Stapleton et al., 2009). At present, they have become the global environmental pollutants. The industrial production of five and eight PBDE products were classified as persistent organic pollutants (POPs) according to "Stockholm Convention" which was amended in 2009.

It was reported that hydroxylated PBDEs (OH-PBDEs) will generate higher toxicity once entering the human or animal body by the catalytic conversion of some metabolic enzymes (Marsh et al., 2004). Similar to PBDEs, the main adverse effects of OH-PBDEs are endocrine disruptors, such as interference with thyroid hormone homeostasis, interference with generation of steroids and toxic neuronal function. Compared with BDE-47, the binding capacity of OH-PBDEs with thyroid hormone transport protein has enhanced several orders of magnitude. 6-OH-BDE-47 has a stronger interference with Ca^{2+} homeostasis and neurotransmitter release capability than BDE-47 (Dingemans et al., 2008).

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In addition, our previous findings indicated that the inhibition of estrogen metabolism in phase one and phase two by OH-PBDEs was stronger than those of PBDEs (Lai et al., 2012 A and B; Lai and Cai 2012). As a metabolite of PBDEs, OH-PBDEs can be further metabolized to PBDEs-quinones resulting in a higher chemical activity. These active quinone metabolites are capable of reacting with DNA adducts and causing DNA damage (Lai et al., 2011).

GSH is a small molecule of tri-peptides, which widely presents in the living cells. As an important antioxidant and free radical scavenger *in vivo*, it plays a significant role in antagonizing exogenous toxins, scavenging free radicals, regulating immune function and maintenance of cell protein structure, inhibiting of apoptosis etc (Richie et al., 1996). GSH can reduce free radical damage to DNA, effectively repairing the damage and mutations of DNA. Moreover, it could directly combine with certain poisons, effectively detoxify exogenous toxic substances (Floreani et al., 1997; Forman et al., 2009).

Quinones are reactive electrophiles, which can readily undergo Michael addition with some intracellular nucleophiles, such as amino acids, GSH, proteins, and nucleic acids (McLean et al., 2000). Rao reported the formation of binding reaction by GSH and 1,4-benzoquinone in the cell (Rao et al., 1988). The reaction of GSH with polychlorinated biphenyl (PCB) quinones was reported, which revealed that GSH reacted rapidly with PCB quinones (PCB-Qs) via Michael addition (Song et al., 2009). A pathway that Bisphenol A quinone (BPA-Q) reacted with GSH and DNA was elucidated (Qiu et al., 2004). A computational study of the reactivity of BPA-Q with deoxyadenosine and GSH suggested that BPA-Q preferably reacts with GSH and only reacts with DNA when the level of GSH in the cell is low (Kolšek et al., 2013).

To further understand the PBDEs toxicological mechanism, the experiments were designed to study the bonding of GSH with PBDEs-Qs. LC-MS technique was applied to identify its structural information of adducts.

2. Materials and methods

2.1. Chemicals and reagents

L-GSH and 2,4-dibromophenol were obtained from Sigma (St. Louis, MO, USA). 2-Bromobenzoquinone (2BrBQ) was obtained from Tokyo Chemical Industrial Co., Ltd. (Tokyo, Japan). 2,6-Bromobenzoquinone (26BrBQ) was purchased from APIN Chemicals Ltd. (Abingdon, UK). $CDCl_3$, phenol, 4-bromophenol, and 2,4,6-tribromophenol were purchased from Acros Organics (Geel, Belgium). Precoated Thin Layer Chromatographic (TLC) plates (DC-Fertigplatten SIL G-25 UV254) were purchased from Macherey–Nagel (D€uren, Germany). Dimethyl sulfoxide (DMSO) in analytical grade was purchased from AJAX Chemicals (Sydney, Australia). Deionized water was purified by employing a Milli-Q reagent water system (Millipore, Billerica, MA, USA).

2.2. Synthesis of PBDE-Qs

A 0.4 mmol phenol or bromophenol in dimethylformamide (DMF) (0.5 mL) were reacted with 0.25 mmol bromobenzoquinone in DMF (1 mL) to synthesized PBDE-Qs. The reaction was initiated by adding 0.25 mmol Na_2HPO_4 and 0.08 mmol K_2CO_3.The reaction mixture was constantly stirred at room temperature for 3 h and then was transferred into deionized water. The reaction product was extracted with ethyl acetate. The organic layer was partitioned with deionized water and brine, dried with Na_2SO_4 and finally removed into vacuum. The crude extract was further purified on a TLC plate (CH_2Cl_2-hexane, v:v, 1:1) and purified sample was characterized by NMR and GC–MS.

2.3. Reaction of GSH with PBDE-Qs

The reaction of PBDE-Qs with reduced GSH was conducted as follows.

A 10 μL of GSH solution (0.6 μmol, in distilled water) was reacted with 10 μL of PBDE-Qs solution (0.6 μmol, in DMSO) at 300 μL of ammonium acetate (50 mM, pH = 7.0). The mixture was kept at 37 °C for 1 h in a shaking water bath. The crude extract was purified by passing it through a C-18 solid phase extraction column to remove unreacted GSH and quinone metabolite and followed by activation C18 column with 2 mL of methanol and 2 mL of water, loaded sample onto a C18 column. Elute columns with 1 mL of methanol to obtain products after elute it with 1 mL of water. Collect the eluent and make up the volume to 2 mL, and injected 20 μL for chromatographic analysis.

2.4. ESI-MS/MS and UPLC–MS analysis

The product-ion MS/MS spectrum was recorded from a Bruker Esquire 4000 ion trap mass spectrometer (Bruker-Fransen, Bremen, Germany) equipped with an ESI source. An HP 1100 liquid chromatograph (Hewlett–Packard, Wilmington, DE, USA) equipped with a Waters Symmetry reserved-phase C18 column (2.1 mm × 100 mm, 3.5 mm) was used for the LC separation. The mobile phase consisted of phase A (deionized water) and phase B, acetonitrile (ACN). The solvent gradient was initiated with A/B (98:2, v/v) at a flow rate of 0.15 mL min^{-1}. A volume of 10 μL of each sample was injected into the HPLC system. The first 3 min of effluent was diverted to waste to minimize contamination of the ESI source. Nebulizer gas, flow rate and temperature of dry gas, the ion spray voltage, compound stability and trap drive level were set at 40 psi, 9.0 L min^{-1}, 300 C and 4500 V, 80% and 100%, respectively. All data were acquired in positive ion mode with scanning mass range between *m*/*z* 100 and 1300. The fragmentation amplitude was optimized according to the stability of the GSH adducts.

The quantitative analysis of GSH adducts was conducted by a triple-quadrupole mass spectrometry equipped with an ESI source (Waters AC-QUITY TQ Detector, Waters Corporation, Milford, MA, USA). The PBDE-GSH adducts were separated by an UPLC (Waters ACQUITY UPLC system, Waters Corporation, Milford, MA, USA) with a reversed-phase ethylene bridged hybrid phenyl column (2.1 mm × 150 mm, 1.7 μm). The mobile phase was consisted of A (deionized water) and B (ACN). A gradient program was used as 95% A and 5% B followed by a linear gradient to 65% A and 35% B over the course of 5 min and then a linear gradient to 5% A and 95% B for another 10 min. The column was then allowed to re-equilibrate back to the starting mobile phase of 95% A for 1.0 min, and held the mobile A as 95% for 4 min before the next injection. An injection volume of 10 μL was selected with a flow rate of 0.3 mL min^{-1}. The optimized MS parameters were described as follows: the capillary voltage was 3000 V; the dwell time was 0.05 s; the extractor voltage was 2.5 V; the temperatures of the negative ESI source and desolvation gas were 118 and 500 C, respectively; the cone gas and the desolvation gas flows were 40 and 650 L h^{-1}, respectively. Instrument operation and data acquisition were processed by using the Waters MassLynx V4.1 SCN5 62 software package.

3. Results and discussion

Previous studies indicated that GSH reacted with abundant 1,4-benzoquinone caused an increase in oxidation potentials of reduced GSH, then resulted in enhanced nephrotoxicity (Lau et al., 1988). Therefore, we speculated that PBDEs-Qs metabolites