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Investigation of cysteinyl protein adducts of benzene diolepoxide

Suramya Waidyanatha *, Stephen M. Rappaport

Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400, USA

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Abstract

Metabolism of benzene is fairly complex yielding several reactive metabolites that could form adducts with macromolecules such as DNA and proteins. Systemic availability of these reactive species can be estimated by measuring the corresponding adducts of these species with serum albumin or hemoglobin. We have shown previously that serum albumin adducts of benzene oxide and 1,4-benzoquinone increased in a dose-dependent manner in rodents and humans exposed to benzene. However, formation of macromolecular adducts of other reactive metabolites, such as benzene diolepoxide and *E*,*E*-muconaldehyde, has not received much attention despite the demonstrated mutagenicity and/or carcinogenicity of these metabolites. We studied the reaction of isomers of benzene diolepoxide, namely *syn*-((±)BDE1) and *anti*-BDE ((±)BDE2), with sulfhydryl groups in vitro. The half-life of BDE2 in 0.1 M ammonium acetate buffer (pH 7.6) was (5.46 h) longer that that in serum albumin in the same buffer (3.83 h) indicating the reaction of BDE with nucleophilic sites on albumin. Reaction products between these and L-cysteine, and GSH were identified and characterized by mass spectrometry in an attempt to develop an assay to quantify these adducts in serum albumin.

Keywords: Benzene; Reactive metabolites; Benzene diolepoxide; Protein adducts; Sulfhydryl groups

1. Introduction

Metabolism of benzene is complex, yielding several reactive metabolites that could form adducts with macromolecules such as DNA and proteins within the body (Fig. 1). Systemic availability of these species can be estimated by measuring the corresponding adducts with serum albumin or hemoglobin. We have shown previously that serum albumin adducts of benzene oxide and 1,4-benzoquinone increased in a dose-dependent manner in rodents and humans exposed to benzene [1–3]. However, formation of macromolecular adducts of other metabolites, such as benzene diolepoxide (BDE) and muconaldehyde, has not received much attention despite the demonstrated mutagenicity and/or carcinogenicity of these metabolites [4–9].

BDEs arise from benzene oxide, the initial metabolite of benzene, via the dihydrodiol pathway and

* Corresponding author.
E-mail address: suramya@unc.edu (S. Waidyanatha).

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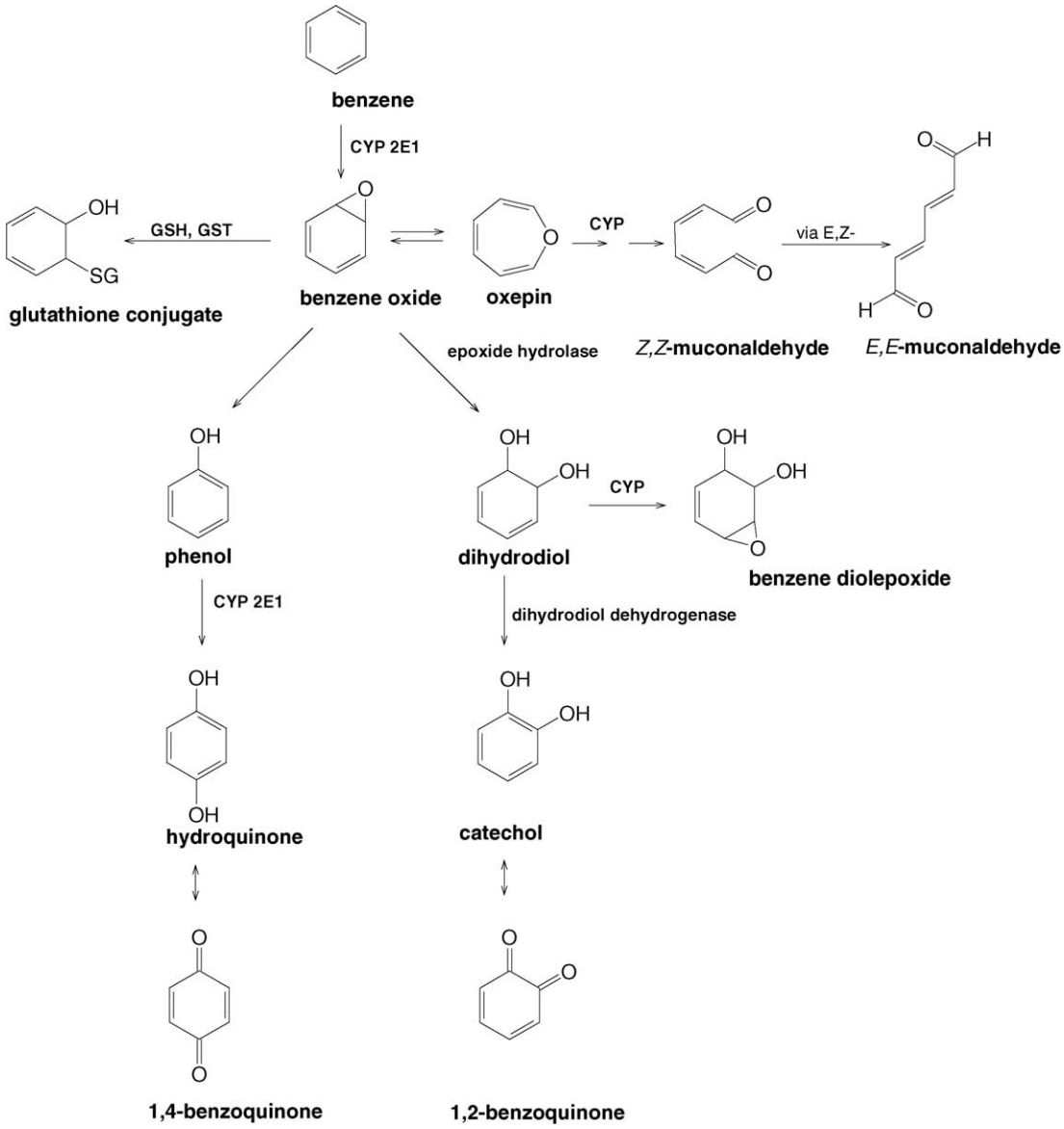

Fig. 1. Metabolism of benzene showing reactive metabolites.

a second P450 oxidation (Fig. 1). The isomers of BDE are designated as *syn*-((±)BDE1) and *anti*-BDE ((±)BDE2) (Fig. 2). Although no direct evidence is available for the formation of these diolepoxides from benzene, (±)BDE2 has been shown to induce lung tumors in newborn mice [4].

The objective of this was to study the reaction between BDE isomers and sulfhydryl groups in an attempt to develop an assay to quantify these adducts in blood proteins. We synthesized racemic BDE1 and BDE2 as shown in Fig. 2 and studied the reactions between these isomers and sulfhydryl groups on cys-

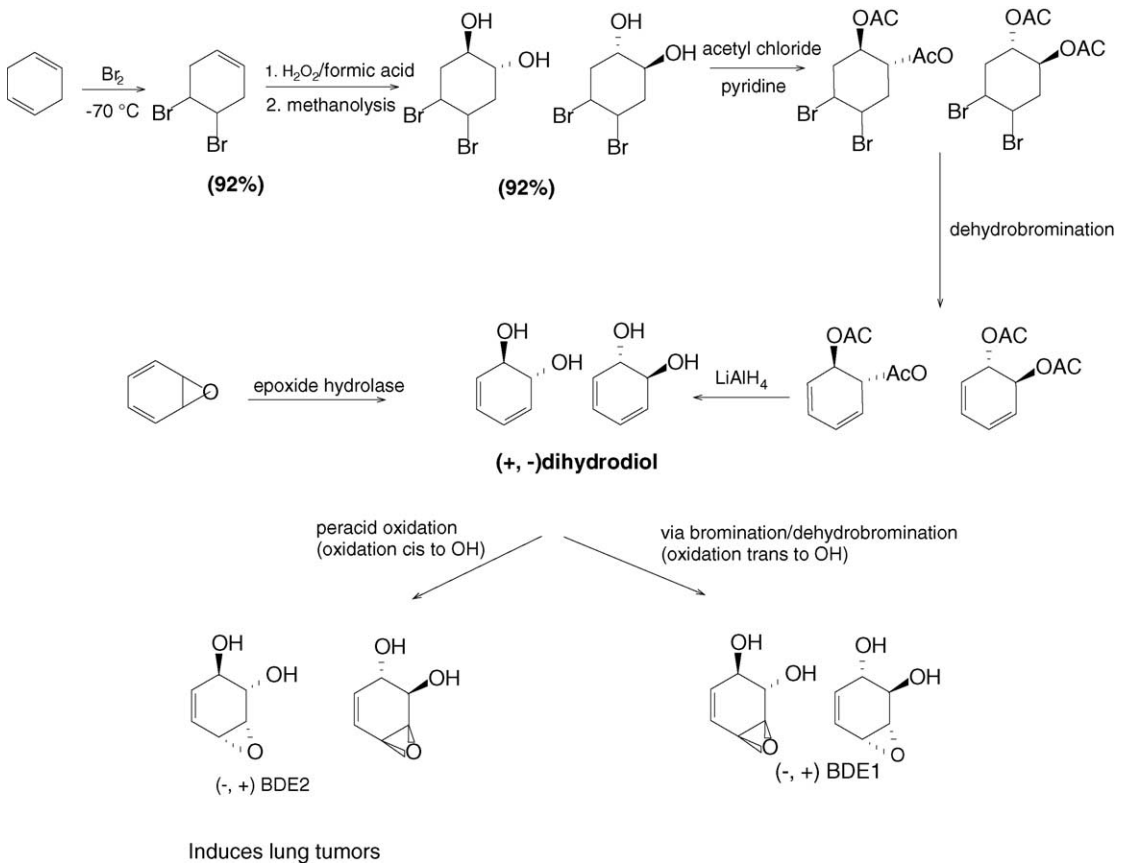

Fig. 2. Scheme for the synthesis of (±)BDE1 and (±)BDE2 [10,11].

teine (BDE-Cys), glutathione (BDE-Glu) and albumin (BDE-Alb).

2. Methods

2.1. Stability of (±)BDE2 in aqueous media

To 1 ml aliquots of either 0.1 M ammonium acetete (pH 7.6) or 25 mg/ml albumin in 0.1 M ammonium acetate (pH 7.6) was added (±)BDE2 in ethyl acetate to give a final concentration of 200 μM BDE2. Samples were reacted for desired times at room temperature and extracted immediately with 2 ml ethyl acetate. Ethyl acetate layers were dried with anhydrous Na_2SO_4 and were brought to dryness under nitrogen. The residue was resuspended in 100 μl hexane, reacted with Trisil® reagent (Pierce, IL) and the corresponding trimethylsilyl derivative was analyzed by GC–EI-MS in selective ion monitoring mode.

2.2. Reaction of (±)BDE2 with L*-cysteine and GSH and characterization of adducts*

(±)BDE2 (7.8 μmol) was reacted overnight at room temperature, with a 1.2 molar excess of either L-cysteine or GSH in 3 ml of 0.1 M ammonium acetete (pH 7.6). Reaction mixtures were analyzed on an LCQDECA quadrupole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with an API2 electrospray ion source operating in the negative mode. Direct injections were performed by introducing 5 μl of each sample into the mass spectrometer to obtain full scans as well as product ions. The MS/MS

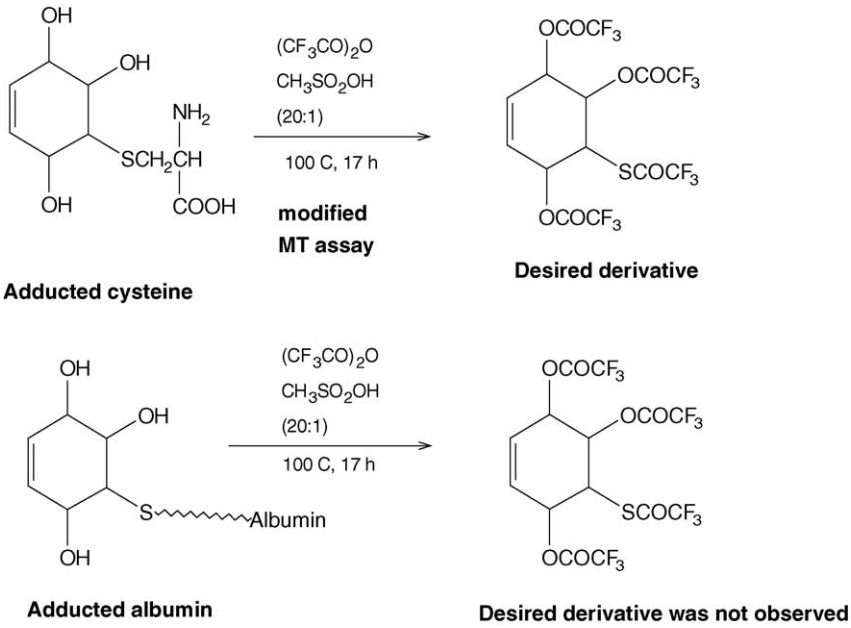

Fig. 3. Reaction of BDE-Cys and BDE-Alb with methanesulfonic acid and trifluoroacetic anhydride releases the trifluoroacetylated derivatives for GC–MS.

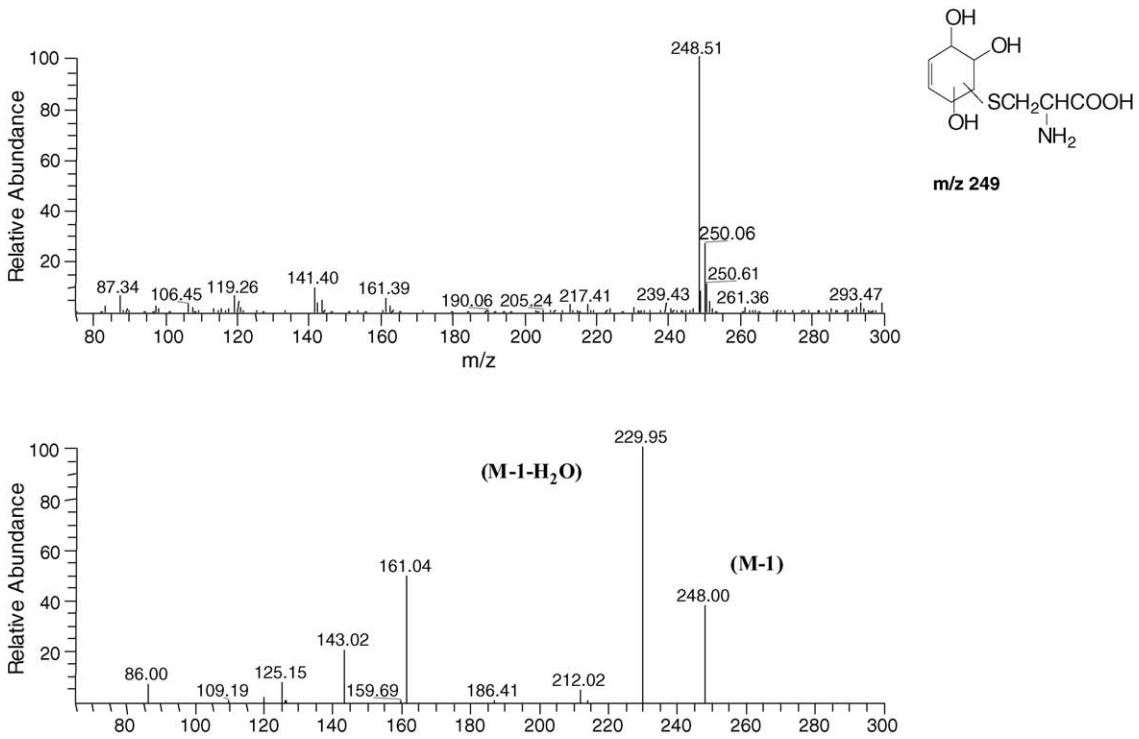

Fig. 4. ESI–MS and MS/MS spectrum of BDE-Cys adduct.

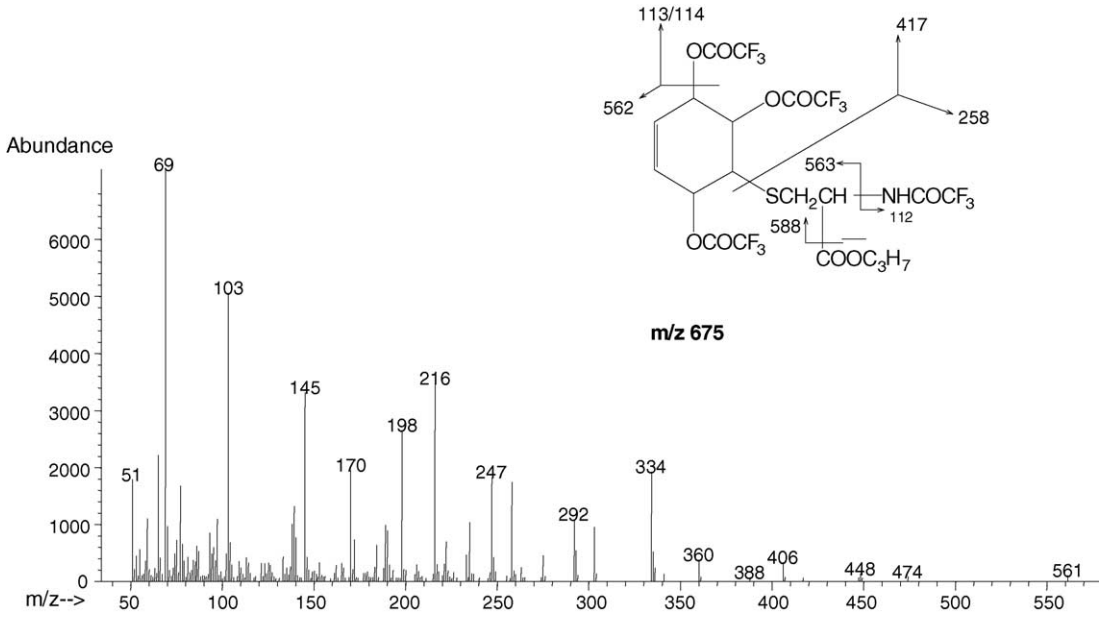

Fig. 5. GC–EI-MS of cys derivative of (±)BDE2.

collision energy was 35 V and the argon pressure was 2.5 mTorr.

2.3. Reaction of BDE2-Cys, BDE2-Glu and BDE-Alb with methanesulfonic acid and trifluoroacetic anhydride (MT assay)

(BDE2-Cys, BDE2-Glu and BDE2-Alb were reacted using the MT assay as described elsewhere for measuring benzoquinone and benzene oxide adducts [12]. Existing conditions were modified to enhance the cleavage of BDE adducts as shown in Fig. 3. Samples were analyzed by GC–NICI- and EI-MS using HP 5890 GC coupled to a 5989 B MS engine.

3. Results and discussion

(±)BDE2 is fairly stable in 0.1 M ammonium acetete (pH 7.6) (half-life 5.46 h) giving a suitable medium to study the reaction between this diolepoxide and nucleophiles. Half-life of (±)BDE2 in in serum albumin in the same buffer was 3.83 h indicating the reaction of (±)BDE2 with nucleophilic sites on albumin. ESI/MS and MS/MS confirms the formation of adducts of (±)BDE2 with L-cysteine and GSH (Fig. 4).

Reaction of BDE-Cys with methanesulfonic acid and trifluoroacetic anhydride under modified MT assay conditions released a derivative, in moderate yields, suitable for GC–MS. However, BDE-Glu and BDE-Alb gave poor yields of this derivative and hence is not a suitable method to quantify these adducts (Fig. 3).

We looked into an alternate approach involving enzyme hydrolysis to release the adducted cysteine followed by derivatization and GC–MS to detect the final derivatives. Initial investigation of digestion of BDE2-Glu with pronase E and subsequent derivatization and GC–MS of the adducted cysteine shows the expected product (Fig. 5). Chromatogram shows two major and two minor products due to nucleophilic attack by sulfhydrl group on both carbons of the epoxide ring of (±)BDE2.

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