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# Mutagenicity of a Glutathione Conjugate of Butadiene Diepoxide

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## Abstract

The mutagenicity and carcinogenicity of the important commodity chemical 1,3-butadiene are attributed to the epoxide products. We confirmed our previous work showing that expression of rat glutathione (GSH) transferase 5-5 enhances the mutagenicity of butadiene diepoxide in *Salmonella typhimurium* TA1535. A GSH-butadiene diepoxide was isolated and fully characterized by mass spectrometry and NMR as *S*-(2-hydroxy-3,4-epoxybutyl)GSH. The conjugate had a $t_{1/2}$ of 2.6 h (pH 7.4, 37 °C) and was considerably more mutagenic than butadiene diepoxide or monoepoxide in *S. typhimurium*. We propose that the GSH conjugate may be a major species involved in butadiene genotoxicity, not a detoxication product.

1,3-Butadiene is an important commodity chemical in industry, particularly in the production of synthetic rubber and other polymers. In 2008, $1.3 \times 10^7$ kg were produced in the United States alone (1). Butadiene is of concern in toxicology because the compound is carcinogenic in mice and rats, particularly the former species (2). In addition, some epidemiology studies suggest that butadiene is a human carcinogen (1, 3). The metabolism of butadiene is understood in the pathways shown in Scheme 1, and it is highly likely that one or more of the epoxide products of butadiene are responsible for its carcinogenicity, probably due to genotoxic mechanisms (4, 5). At least 23 adducts of DNA bases have been reported, with the number being even higher when stereochemistry is considered (6-13).

We previously reported that the base pair mutagenicity of butadiene diepoxide in *Salmonella typhimurium* TA1535 is considerably increased by the cellular expression of rat GSH transferase 5-5 (14). That result, unexpected at the time, has been repeatable (Supporting Information Figure S1) and implies that a GSH conjugate is genotoxic. GSH conjugation has been reported but the exact structure of the conjugate was not determined (15, 16). However, GSH conjugation has usually been considered to be a detoxication process in the metabolism of butadiene diepoxide (17).

A GSH-butadiene diepoxide conjugate (**1**) was prepared by either (i) reacting butadiene diepoxide with GSH in $CH_3OH$ in the presence of 3 equivalents of Na° or $NaOCH_3$ (18) or (ii) enzymatic reaction of butadiene diepoxide (2 mM) with GSH (5 mM) in the presence of a commercial mixture of rat liver GSH transferase (Sigma, St. Louis, MO; 0.1 mg $mL^{-1}$) in 0.10 M Tris-HCl buffer (pH 7.7) for 1 h at 37 °C. The latter approach yielded less complex products and was used in the work presented here. LC-MS analysis showed the formation of a peak with the features expected for a GSH conjugate (Supporting Information Figure S2),

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Supporting Information **Available:** GSH transferase-dependent mutagenicity of butadiene diepoxide in *S. typhimurium* TA1535, LC-MS characterization of GSH-butadiene diepoxide conjugate, NMR spectra of GSH-butadiene diepoxide conjugate, and MS and NMR spectra of butadiene diol epoxide. This material is available free of charge via the Internet at http://pubs.acs.org.

and high resolution analysis yielded an $MH^+$ ion at *m/z* 394.1276 (calc. for $C_{14}H_{24}N_3O_8S$, 394.1284, -2.0 ppm). Of the HPLC peaks (Supporting Information Figure S3), only one gave a positive colorimetric assay with *p*-nitrobenzylpyridine reagent (19), indicating the presence of an electrophile. The conjugate was isolated in preparative incubations, frozen, and lyophilized. The $t_{½}$ was determined in 0.10 M potassium phosphate buffer (pH 7.4) at 37 °C and was 2.6 ± 0.1 h (Figure 1). The structure was established by a combination of NMR methods ($^1H$, $^{13}C$, total correlation spectroscopy (TOCSY), heteronuclear multiple bond correlation (HBMC), heteronuclear single quantum correlation (HSQC); Supporting Information Figures S4-S8) and is shown as **1** in Scheme 1. In particular, GSH attack at C-2 is ruled out (**2**), as is structure **3**. The product was quantified using $^1H$ NMR and an internal standard (benzene). An $\varepsilon_{560}$ value of 6.57 $mM^{-1}$ $cm^{-1}$ was determined for the *p*-nitrobenzylpyridine assay based on the $^1H$-NMR signals (which can be used for convenient determination of stock concentrations).

The conjugate, *S*-(2-hydroxy-3,4-epoxybutyl)GSH, was considerably more mutagenic than several other related epoxides in *S. typhimurium* TA1535, a tester strain that reports base pair mutations at a GC site (Figure 2). Other compounds tested included butadiene monoepoxide, butadiene diepoxide, and butadiene diol epoxide (prepared by reaction of 3,4-dihydroxybutene with *m*-chloroperbenzoic acid, Supporting Information Figures S9, S10). These results, along with the enhancement of butadiene diepoxide by GSH transferase expression, argue for a prominent role of the characterized GSH-butadiene diepoxide conjugate in butadiene genotoxicity.

Although many butadiene-derived adducts of DNA bases have been prepared (6-13), limited information is available about their occurrence in biological systems and/or their biological activities. We show a role for a defined GSH conjugate of butadiene diepoxide in (base pair) mutagenicity (Figure 2), congruent with previous results on the role of GSH conjugation in butadiene bioactivation (14, 20) (Supporting Information Figure S1). We have not yet evaluated the mutagenicity and other properties of *S*-(2-hydroxy-3,4-epoxybutyl)GSH in mammalian systems, which will be of interest. Preliminary LC-MS studies have shown the formation of Gua and Ade adducts when the GSH-butadiene diepoxide conjugate was incubated with DNA or DNA bases (results not presented). Future studies are focused on characterization of the DNA adducts formed from GSH-butadiene diepoxide, identification of which of these are miscoding, and analysis of these adducts *in vivo*. Three stereoisomers of butadiene diepoxide (*R*,*R*, *S*,*S*, and *meso*) are known and some differences have been reported for their rates of formation and reactivity (21-25) but we have not prepared the corresponding conjugates yet. The GSH transferase selectivity of the conjugation reaction also remains to be established. Some of the work involved rat and human theta-class enzymes (14, 20) (Supporting Information Figure S1) but a mixture of other rat GSH transferases could be used, as shown in the preparative work.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## Abbreviations

| | |
|---|---|
| **HBMC** | heteronuclear multiple bond correlation |
| **HSQC** | heteronuclear single quantum correlation |
| **TOCSY** | total correlation spectroscopy |

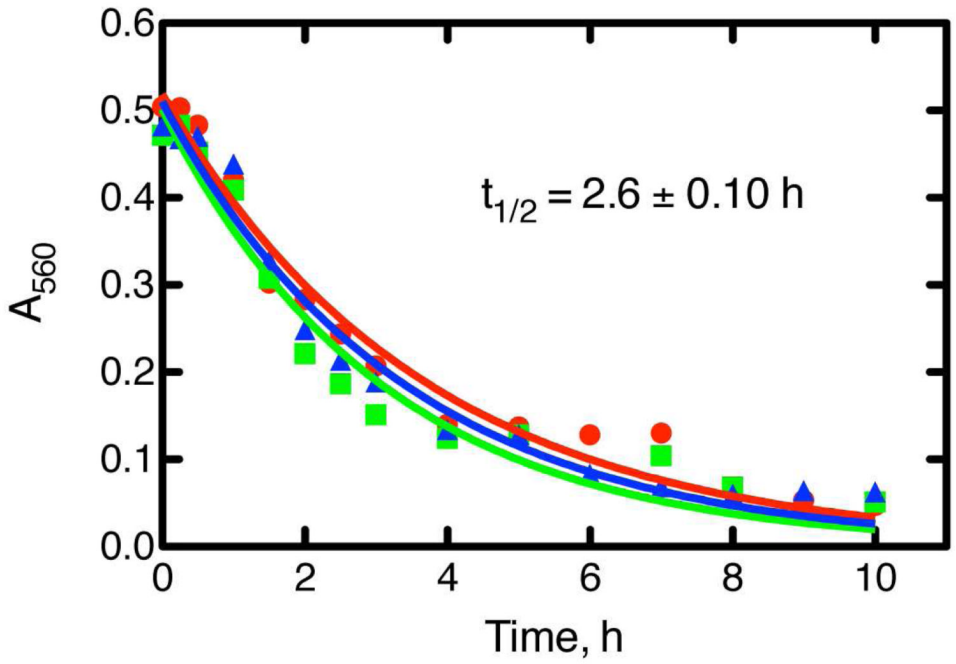



**Figure 1.**
Determination of the $t_{1/2}$ of the purified GSH-butadiene diepoxide conjugate using a *p*-nitrobenzylpyridine assay (18, 19). The results shown are from three separate experiments (separate symbols), with the mean $t_{1/2}$ ± SD indicated.

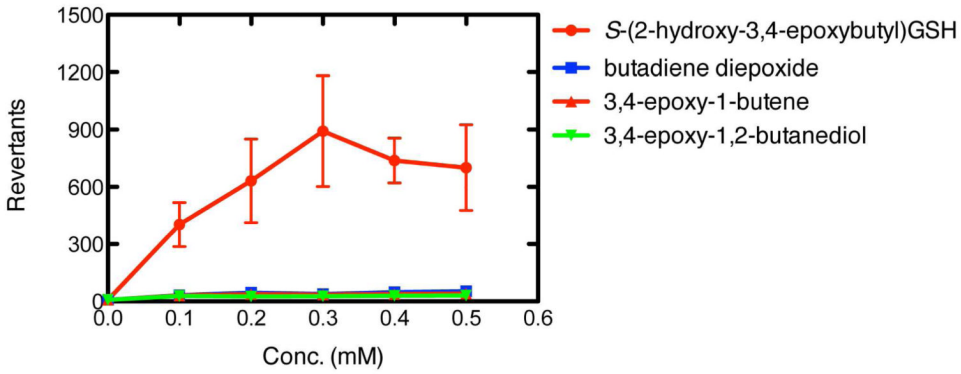
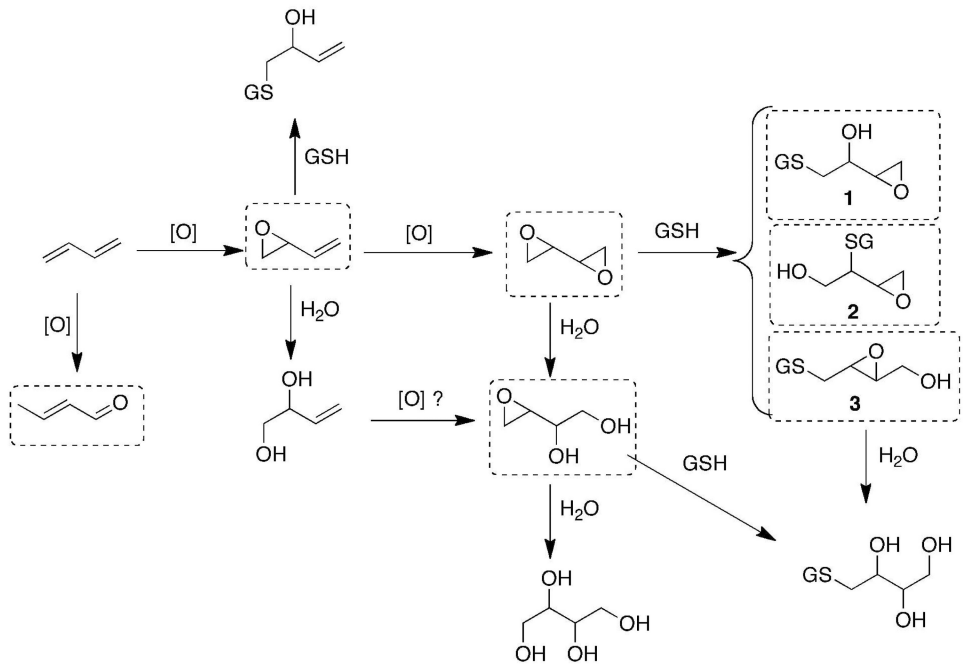



**Figure 2.**

Mutagenicity of GSH-butadiene diepoxide conjugate in *S. typhimurium* TA1535. Direct comparisons were made with the indicated other three epoxides, using the methods described in (14). Results are shown as means and ranges obtained for duplicate assays.




**Scheme 1.**
Metabolism of butadiene. Three possible GSH adducts formed from butadiene diepoxide are shown. All compounds in dotted boxes can potentially react with DNA.