# Location of the epoxide function determines specificity of the allelic variants of human glutathione transferase Pi toward benzo[*c*]chrysene diol epoxide isomers

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**Abstract Carcinogenic activity of many polycyclic aromatic hydrocarbons (PAHs) is mainly attributed to their respective diol epoxides, which can be classified as either bay or fjord region depending upon the location of the epoxide function. The Pi class human glutathione (GSH) transferase (hGSTP1-1), which is polymorphic in humans with respect to amino acid residues in positions 104 (isoleucine or valine) and/or 113 (alanine or valine), plays an important role in the detoxification of PAH-diol epoxides. Here, we report that the location of the epoxide function determines specificity of allelic variants of hGSTP1-1 toward racemic *anti*-diol epoxide isomers of benzo[*c*]chrysene (B[*c*]C). The catalytic efficiency ($k_{cat}/K_m$) of V104,A113 (VA) and V104,V113 (VV) variants of hGSTP1-1 was approximately 2.3- and 1.7-fold higher, respectively, than that of the I104,A113 (IA) isoform toward bay region isomer (±)-*anti*-B[*c*]C-1,2-diol-3,4-epoxide. On the other hand, the IA variant was approximately 1.6- and 3.5-fold more efficient than VA and VV isoforms, respectively, in catalyzing the GSH conjugation of fjord region isomer (±)-*anti*-B[*c*]C-9,10-diol-11,12-epoxide. The results of the present study clearly indicate that the location of the epoxide function determines specificity of the allelic variants of hGSTP1-1 in the GSH conjugation of activated diol epoxide isomers of B[*c*]C.** © **2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

*Key words:* Benzo[*c*]chrysene; Carcinogenesis; Glutathione transferase Pi; Polymorphism; Detoxification

## 1. Introduction

Many polycyclic aromatic hydrocarbons (PAHs) are human carcinogens that are widespread in the environment. Tumorigenic activity of PAHs is mainly due to their respective diol epoxides, which are formed through mediation of cytochrome P450-dependent monooxygenases and epoxide hydrolase [1]. The Pi class human glutathione (GSH) transferase (hGSTP1-1) is believed to play an important role in the detoxification of PAH-diol epoxides primarily by catalyzing their conjugation with GSH [2,3]. Interestingly, the *hGSTP1* gene is polymorphic in human populations. The allelic variants of hGSTP1-1 differ at amino acid residues in positions 104 (isoleucine or valine) and/or 113 (alanine or valine) [4–6] and exhibit marked differences in their activities toward the model substrate 1-chloro-2,4-dinitrobenzene [6]. Epidemiological studies have shown that while *I104,A113* allele is the most frequent in human populations [7–10], the frequency of the *V104,A113* and (or) *V104,V113* alleles is significantly higher in certain cancers compared with the *I104,A113* allele [5,7,8].

To gain insights into the toxicological relevance of hGSTP1-1 polymorphism, we have previously examined the specificities of the allelic variants of this enzyme toward carcinogenic *anti*-diol epoxide isomers of various environmentally relevant PAHs, including benzo[*a*]pyrene (B[*a*]P), chrysene, 5-methylchrysene (5-MeC), benzo[*g*]chrysene (B[*g*]C), and benzo[*c*]phenanthrene (B[*c*]P) [11–14]. Our studies show that the V104,A113 and V104,V113 variants of hGSTP1-1 (VA and VV, respectively) are between 1.3- and 7-fold more efficient than the I104,A113 isoform (IA) toward (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide ((+)-*anti*-BPDE) and (±)-*anti*-chrysene-1,2-diol-3,4-epoxide ((±)-*anti*-CDE) [11,12]. Surprisingly, the above ranking of catalytic efficiencies of hGSTP1-1 variants is reversed toward certain other environmentally relevant carcinogenic PAH-diol epoxides, including (+)-*anti*-5-methylchrysene-1,2-diol-3,4-epoxide ((+)-*anti*-5MeCDE); (±)-*anti*-benzo[*g*]chrysene-11,12-diol-13,14-epoxide ((±)-*anti*-B[*g*]CDE), and (±)-*anti*-benzo[*c*]phenanthrene-3,4-diol-1,2-epoxide ((±)-*anti*-B[*c*]PDE), where the IA variant is relatively more efficient than VA and/or VV [13,14].

The diol epoxides implicated as the active metabolites of PAH carcinogens generally have their epoxide function either in a bay region or in a fjord region [1,15]. While *anti*-diol epoxide isomers of the bay region class, e.g. *anti*-BPDE, are planar molecules, *anti*-diol epoxide isomers of the fjord region class, such as *anti*-B[*g*]CDE, have their epoxide function in a sterically-hindered region, and their molecules are distorted from planarity [1,15]. These results suggest that while VA and/or VV isoforms may play a major role in the GSH conjugation of planar bay region PAH-diol epoxides (e.g. *anti*-BPDE), the IA isoform is likely to be important in the detoxification of non-planar and sterically-hindered fjord region

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compounds such as *anti*-B[*g*]CDE [11–14]. In the present study we have tested the above hypothesis by determining catalytic efficiencies of the allelic variants of hGSTP1-1 toward racemic *anti*-benzo[*c*]chrysene-1,2-diol-3,4-epoxide ((±)-*anti*-B[*c*]CDE-1) and racemic *anti*-benzo[*c*]chrysene-9,10-diol-11,12-epoxide ((±)-*anti*-B[*c*]CDE-2), which are the activated forms of benzo[*c*]chrysene (B[*c*]C) [16]. B[*c*]C is intriguing due to the presence of both, a bay region and a fjord region in the same molecule. As shown in Fig. 1, the epoxide function in the (±)-*anti*-B[*c*]CDE-1 isomer is in a bay region, whereas (±)-*anti*-B[*c*]CDE-2 has fjord region characteristics due to the location of its epoxide function in a sterically-hindered region. In this communication we report that the location of the epoxide function determines catalytic efficiency of the allelic variants of hGSTP1-1 toward (±)-*anti*-B[*c*]CDE isomers.

## 2. Materials and methods

### 2.1. Expression and purification of allelic variants of hGSTP1-1

The hGSTP1-1 variants were expressed and purified by GSH affinity chromatography as described previously [11].

### 2.2. Determination of GST activity toward (±)-anti-B[c]CDE isomers

The (±)-*anti*-B[*c*]CDE isomers were synthesized and purified as described previously [17]. The reaction mixture in a final volume of 0.1 ml consisted of Tris/HCl buffer (pH 7.5) containing 2.5 mM KCl and 0.5 mM EDTA (TKE buffer), 2 mM GSH, and desired concentrations of (±)-*anti*-B[*c*]CDE-1 or (±)-*anti*-B[*c*]CDE-2 and hGSTP1-1 isoform protein. The reaction was initiated by adding the diol epoxide substrate, and the reaction mixture was incubated for 30 s at 37°C. The reaction was stopped by rapidly adding 0.1 ml of chilled acetone, and the unreacted (±)-*anti*-B[*c*]CDE was removed by extracting the reaction mixture with ethyl acetate saturated with TKE buffer. The GSH conjugate of (±)-*anti*-B[*c*]CDE-1 or (±)-*anti*-B[*c*]CDE-2 in the aqueous phase was quantified by reverse-phase HPLC using a Waters Nova-Pak $C_{18}$ (3.9×150 mm) column. This column was preequilibrated with 80% solvent I (5% acetonitrile in 0.1% trifluoroacetic acid) and 20% solvent II (90% acetonitrile in 0.1% trifluoroacetic acid). The GSH conjugates of *anti*-B[*c*]CDE-1 and *anti*-B[*c*]CDE-2 were eluted with a 20–30% linear gradient of solvent II in 20 min at a flow rate of 1 ml $min^{-1}$. Under these conditions, the GSH conjugates of *anti*-B[*c*]CDE-1 and *anti*-B[*c*]CDE-2 were eluted at retention times of about 12 and 7.5 min, respectively. Initially, the GST activity was measured as a function of varying hGSTP1-1 protein concentration to optimize the assay conditions. In subsequent studies, 60 and 120 μg $ml^{-1}$ of hGSTP1-1 isoform protein were used for incubations involving *anti*-B[*c*]CDE-1 and *anti*-B[*c*]CDE-2, respectively. For (±)-*anti*-B[*c*]CDE-1, the GST activity was measured as a function of varying diol epoxide concentration (10–320 μM) at a fixed saturating concentration of GSH (2 mM) to determine the kinetic parameters. The $K_m$, $V_{max}$, and $k_{cat}/K_m$ (catalytic efficiency) values were determined by non-linear regression analysis of the experimental data points. The catalytic efficiencies of hGSTP1-1 variants toward (±)-*anti*-B[*c*]CDE-2 were calculated from initial rate measurements at low (10 μM) substrate concentrations, as described in [18], because saturation curves could not be obtained for this isomer.

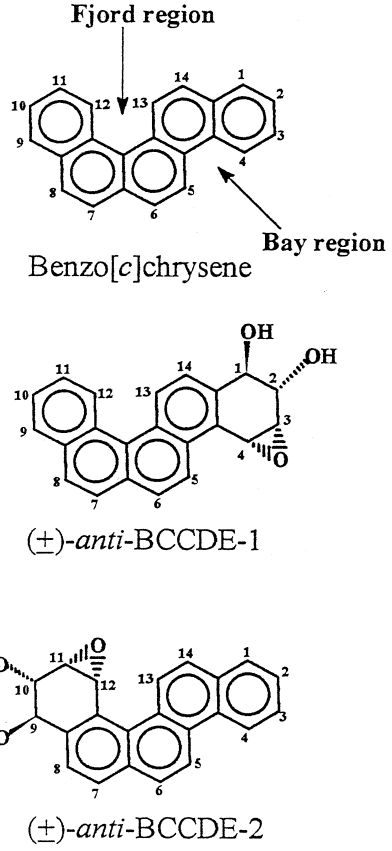


Fig. 1. Structures of benzo[*c*]chrysene and its bay region ((±)-*anti*-B[*c*]CDE-1) and fjord region ((±)-*anti*-B[*c*]CDE-2) diol epoxide isomers.

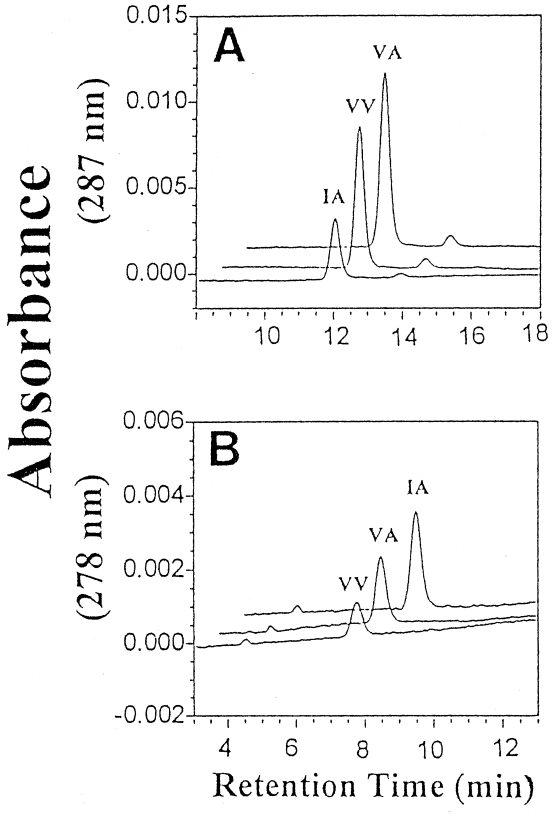


Fig. 2. Reverse-phase HPLC analysis of water-soluble products resulting from the reaction of 2 mM GSH with (A) 10 μM (±)-*anti*-B[*c*]CDE-1, and (B) 10 μM (±)-*anti*-B[*c*]CDE-2 in the presence of hGSTP1-1 protein. The concentrations of hGSTP1-1 variants were 60 μg $ml^{-1}$ and 120 μg $ml^{-1}$ for (±)-*anti*-B[*c*]CDE-1 and (±)-*anti*-B[*c*]CDE-2, respectively.

## 3. Results and discussion

Fig. 2A exemplifies a reverse-phase HPLC elution profile of

water-soluble products resulting from the reaction of 2 mM GSH and 10 μM (±)-*anti*-B[*c*]CDE-1 for 30 s in the presence of allelic variants of hGSTP1-1. Non-enzymatic (spontaneous) conjugation of (±)-*anti*-B[*c*]CDE-1 with GSH was not detectable (data not shown in Fig. 2A). The levels of GSH conjugate of *anti*-B[*c*]CDE-1 in the reaction mixtures containing hGSTP1-1 variants VA and VV exceeded those observed with IA by about 2.8- and 1.6-fold, respectively. Interestingly, as shown in Fig. 2B, the IA variant, not VA or VV, was found to be most effective in catalyzing the GSH conjugation of (±)-*anti*-B[*c*]CDE-2. Non-enzymatic GSH conjugation of (±)-*anti*-BCCDE-2 was also not detectable (data not shown in Fig. 2B).

All three variants of hGSTP1-1 adhered to Michaelis–Menten kinetics toward (±)-*anti*-B[*c*]CDE-1 (data not shown). Table 1 summarizes the kinetic constants for hGSTP1-1 variants toward (±)-*anti*-B[*c*]CDE-1. The $V_{max}$ values for the VA and VV isoforms did not differ from each other, but both were significantly higher (approximately 6.7-fold) than that for the IA variant. While the $K_m$ for the VV isoform was significantly higher (approximately 4.0-fold) than for the IA isoform, this parameter did not differ significantly between either IA and VA or VA and VV variants. Of the three variants, VA was found to be most efficient in catalyzing the GSH conjugation of (±)-*anti*-B[*c*]CDE-1. While VA was approximately 2.3-fold more efficient catalytically than IA ($P \approx 0.05$), the catalytic efficiencies of VA and VV were not significantly different from each other. Even though the VV isoform was about 1.7-fold more efficient than IA in catalyzing the GSH conjugation of (±)-*anti*-B[*c*]CDE-1, this difference did not reach statistical significance.

Unlike (±)-*anti*-B[*c*]CDE-1, concentrations of (±)-*anti*-B[*c*]CDE-2 approaching saturation of the enzyme could not be attained (data not shown). Therefore, catalytic efficiencies toward (±)-*anti*-B[*c*]CDE-2 were calculated from the initial reaction rate measurements at 10 μM substrate concentration, as described by us previously [14]. As shown in Fig. 3, the IA variant of hGSTP1-1 was found to be most efficient in catalyzing the GSH conjugation of the (±)-*anti*-B[*c*]CDE-2 isomer. The catalytic efficiency of the IA isoform was significantly higher ($P = 0.001$) than that of either VA (about 1.6-fold) or VV (about 3.5-fold). The catalytic efficiencies of VA and VV toward (±)-*anti*-B[*c*]CDE-2 were also statistically significantly different from each other at $P = 0.001$.

We were the first to report that allelic variants of hGSTP1-1 significantly differ in their ability to catalyze the GSH conjugation (detoxification) of the ultimate carcinogenic metabolites (diol epoxides) of certain PAHs [11–14]. We found that hGSTP1-1 variants with valine in position 104 (VA and VV) are relatively more efficient than the IA isoform toward (+)-*anti*-BPDE [11,12], which is a planar PAH-diol epoxide. Our observations on differences in catalytic efficiencies between hGSTP1-1 variants toward (+)-*anti*-BPDE have been independently confirmed by other investigators [19]. Subsequently, we showed that the hGSTP1-1 variants with valine in position 104 are significantly more efficient than the IA isoform in catalyzing the GSH conjugation of (±)-*anti*-CDE [11,12], which, like (+)-*anti*-BPDE, is a planar molecule. Surprisingly, the IA isoform, not VA or VV, was found to be most effective toward (±)-*anti*-B[*g*]CDE and (±)-*anti*-B[*c*]PDE [14]. One common feature among these diol epoxides is that their epoxide group is in a sterically-hindered region, and these molecules are distorted from planarity. Based on these observations, we predicted that while VA and/or VV isoforms may play a major role in the detoxification of bay region diol epoxides, the IA variant is likely to be important in the GSH conjugation of fjord region PAH-diol epoxides.

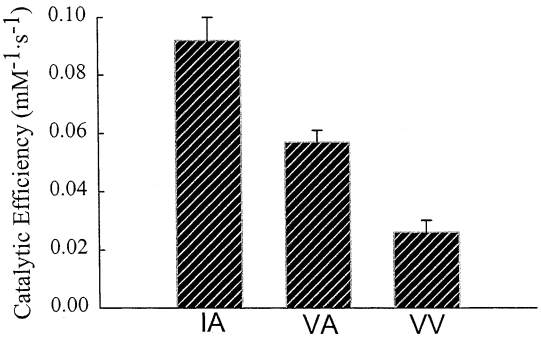


Fig. 3. Catalytic efficiencies of hGSTP1-1 variants toward (±)-*anti*-B[*c*]CDE-2. Data are means ± S.E.M. of three determinations. The catalytic efficiency values were calculated from initial reaction rates at 10 μM substrate concentration. One-way ANOVA followed by multiple comparisons with appropriate corrections (Duncan, Scheffe) reveals that the catalytic efficiency of every isoenzyme is different from the other two at $P = 0.001$.

B[*c*]C, a constituent of coal tar and crude oil, presented us with a unique opportunity to test the above hypothesis since this environmental pollutant can be activated to both a bay region as well as a fjord region diol epoxide (Fig. 1). The results of the present study clearly demonstrate that the VA and VV isoforms are more efficient than IA toward (±)-*anti*-B[*c*]CDE-1, which is a bay region class diol epoxide. On the other hand, the IA isoform is significantly more efficient than either VA or VV toward the (±)-*anti*-B[*c*]CDE-2 isomer, which has fjord region characteristics due to the location of its epoxide function in a sterically-hindered region.

Sundberg et al. [20] have also compared the efficiencies of the IA and VA variants of hGSTP1-1 in catalyzing the GSH

Table 1
Kinetic constants for hGSTP1-1 variants toward (±)-*anti*-B[*c*]CDE-1

| Kinetic constants | hGSTP1-1 variant | | |
|---|---|---|---|
| | IA | VA | VV |
| $V_{max}$ (nmol mg$^{-1}$ min$^{-1}$) | 16 ± 2[a,b] | 107 ± 21 | 107 ± 20 |
| $K_m$ (μM) | 111 ± 27[b] | 314 ± 106 | 446 ± 126 |
| $k_{cat}$ (s$^{-1}$) | 0.014[a,b] | 0.089 | 0.088 |
| $k_{cat}/K_m$ (mM$^{-1}$ s$^{-1}$) | 0.12 ± 0.02[a] | 0.28 ± 0.04 | 0.20 ± 0.02 |

Values are means ± S.E.M. of three determinations. The kinetic parameters for VA and VV do not differ significantly from each other.
[a]Statistically significantly different from VA.
[b]Statistically significantly different from VV.

conjugation of certain fjord region class PAH-diol epoxides, including optically pure (−)-enantiomers of *anti*-B[*c*]CDE-2 and *anti*-B[*g*]CDE. While VV variant was not examined, the catalytic efficiencies of the IA and VA isoforms of hGSTP1-1 toward (−)-*anti*-B[*g*]CDE were found to be comparable [20]. Previous studies from our laboratory using racemic *anti*-B[*g*]CDE have also shown that the catalytic efficiency of the IA variant (0.36 $mM^{-1}$ $s^{-1}$) is not statistically significantly different from that of VA (0.31 $mM^{-1}$ $s^{-1}$) [14]. On the other hand, our results are different from those of Sundberg et al. [20] with respect to activity of hGSTP1-1 isoforms toward *anti*-B[*c*]CDE-2. The results of the present study reveal that the IA isoform is significantly more efficient than VA in catalyzing the GSH conjugation of *anti*-B[*c*]CDE-2. Unlike our observations, IA and VA were found to be equally effective toward optically pure (−)-*anti*-B[*c*]CDE-2 by Sundberg et al. [20]. In addition, the catalytic efficiency values for hGSTP1-1 variants obtained during the present investigation using racemic *anti*-B[*c*]PDE-2 are relatively lower than those reported by Sundberg et al. [20]. While reasons for these discrepancies are not known, some possibilities exist that may explain these differences. First, it is possible that the (+)-enantiomer of *anti*-B[*c*]CDE-2 acts as an inhibitor of hGSTP1-1 catalyzed GSH conjugation of the (−)-*anti*-B[*c*]CDE-2 isomer if incubations are performed using racemic substrate, which was the case in the present study. In this regard, it is important to point out that the (−)-*anti*-BPDE isomer has been shown to inhibit GSH conjugation of (+)-*anti*-BPDE [2]. Second, the catalytic efficiencies toward racemic *anti*-B[*c*]CDE-2 in the present study are calculated from initial reaction rate measurements at 10 μM substrate concentration since concentrations of (±)-*anti*-B[*c*]CDE-2 approaching saturation of the enzyme could not be attained. On the other hand, Sundberg et al. [20] used concentrations of 40 and 80 μM to determine catalytic efficiencies of hGSTP1-1 variants toward (−)-*anti*-B[*c*]CDE-2. Despite these discrepancies, however, the results of the present study taken together with our earlier findings clearly demonstrate differences in specificities of allelic variants of hGSTP1-1 toward bay- and fjord region class diol epoxides of various environmentally relevant PAH carcinogens [11–14].

In conclusion, the results of the present work, taken together with our previous studies [11–14], indicate that the catalytic efficiency of allelic variants of hGSTP1-1 toward PAH-diol epoxides is dependent upon the location (fjord versus bay region) of the epoxide group in these environmental carcinogens. Our results suggest that the putative role of hGSTP1-1 polymorphism in humans with regard to the susceptibility of individuals to PAH-induced carcinogenesis may be more complex than currently realized.

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