Advances in Brief

Catalytic Efficiencies of Allelic Variants of Human Glutathione S-transferase P1-1 toward Carcinogenic anti-Diol Epoxides of Benzo[c]phenanthrene and Benzo[g]chrysene

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Abstract

Four allelic variants of glutathione (GSH) S-transferase P1-1 (hGSTP1-1) that differ in their structures at amino acid(s) in position(s) 104 and/or 113 are known to exist in human populations. However, the physiological significance of hGSTP1-1 polymorphism is not fully understood. In this communication, we report that the I104,A113 allele of hGSTP1-1, which is most frequent in human populations, is also most efficient in the GSH conjugation of carcinogenic anti-diol epoxides of benzo[g]chrysene and benzo[c]phenanthrene (anti-BGCDE and anti-BCPDE, respectively). The catalytic efficiency of hGSTP1-1(I104,A113) isoform toward anti-BGCDE, 0.36 mmol/min/mg, was ~1.7-fold higher (P < 0.05) compared with hGSTP1-1(V104,V113). Interestingly, the frequency of codon 104-valine alleles is significantly higher in certain cancers compared with codon 104-isoleucine alleles. Like anti-BGCDE, the catalytic efficiency of hGSTP1-1(I104,A113) isoform toward anti-BCPDE was higher by about 1.4- to 2.3-fold (P < 0.05) than those of other hGSTP1-1 variants. These observations are interesting because we have shown previously (Hu, X. et al., Biochem. Biophys. Res. Commun., 238: 397-402, 1997) that the V104,V113 variant, not the I104,A113 isoform, is most efficient in the GSH conjugation of bay-region anti-diol epoxide of benzo(a)pyrene (anti-BPDE), which, unlike anti-BGCDE or anti-BCPDE, is a planar molecule. In conclusion, our results suggest that hGSTP1-1 polymorphism may be an important factor in differential susceptibility of humans to cancers where poly(cyclic aromatic hydrocarbons are etiological factors and that I104,A113 variant may play a major role in the detoxification of nonplanar, sterically hindered fjord-region diol epoxides (e.g., anti-BGCDE).

Introduction

PAHs are widespread environmental pollutants that are abundant in cigarette smoke and other substances and are suspected human carcinogens (1). It is well known that the diol epoxides of the PAHs, rather than the parent compounds, are the ultimate carcinogens of these environmental pollutants (2, 3). Covalent interaction of the PAH-diol epoxide with nucleophilic sites in DNA is an important event in PAH-induced carcinogenesis. Although a number of different mechanisms can detoxify PAH-diol epoxides, the most important mechanism in their detoxification is the GST-catalyzed conjugation with GSH (4-9). Cytosolic GSTs are a superfamily of detoxification isoenzymes that have been categorized into five known major classes—Alpha, Mu, Pi, Theta, and Zeta—based on their physicochemical properties (10-13). Isoenzymes of these classes differ significantly in their substrate specificities (10, 11). For example, the Pi class isoenzyme (hGSTP1-1) is relatively more efficient than other classes of GSTs in the GSH conjugation of anti-BPDE (8), which is the ultimate carcinogen of widespread environmental pollutant benzo(a)pyrene (2, 3).

The hGSTP1-1 gene has been shown to be polymorphic in humans (14-20). Four allelic variants of hGSTP1-1 [hGSTP1-1(I104,A113), hGSTP1-1(I104,V113), hGSTP1-1(V104,A113) and hGSTP1-1(V104,V113)], differing in their structures by the amino acid(s) in position(s) 104 (isoleucine or valine-ATC or GTC in nucleotide position 313-315; including the initiator methionine some publications refer this residue as 105) and/or 113 (alanine or valine-GCG or GTG in nucleotide position 340-342; including the initiator methionine some publications refer this residue as 114) have now been identified in human populations (16-20). These studies have shown that, although I104,A113 allele is most frequent in human populations, the I104,V113 is a rare allele (16-20). The frequency of codon 104 valine has been shown to be significantly higher in cancer patients as compared with codon 104 isoleucine (16, 17, 19, 20). However, the molecular basis and physiological implications of these epidemiological observations are not fully known.

We have demonstrated previously (21) that the allelic variants of hGSTP1-1 differ significantly in their kinetic properties. More recently, we have shown (22, 23) that the hGSTP1-1 forms with valine in position 104 ([V104,A113] and [V104,V113]) are relatively more effective toward (+)-anti-BPDE than the isoenzymes with isoleucine in position 104. Subsequently, Sundberg et al. (24) have confirmed our observations and demonstrated that the hGSTP1-1(V104,A113) variant (referred to as GSTP1-1/V-105 in Ref. 24) is significantly more active than hGSTP1-1 (I104,A113) form (referred to as GSTP1-1/I-105 in Ref. 24) in conjugation reactions with bulky PAH-diol epoxides including anti-BPDE. These observations led us to hypothesize that subjects homozygous for the V104,V113 alleles may be at a relatively lower risk for PAH-induced carcinogenesis than individuals with other hGSTP1-1 genotypes. Recent epidemiological studies, however, have shown that the codon 104 valine alleles, not the I104,A113 allele, is strongly associated with various cancers including bladder, testicular, and breast cancer (16, 17, 19, 20). Although BPDE is the prototypical and best studied diol epoxide, other members of this family such as BGCDE and BCPDE are potent carcinogens and occur in environment at comparable levels (1, 25, 26). Therefore, to understand the role of hGSTP1-1 polymorphism in PAH-induced carcinogenesis, it is essential that the speci-
ficiencies of hGSTP1-1 variants toward diol epoxides other than BPDE be determined.

In this communication, we report specificities of hGSTP1-1 variants toward racemic anti-BGCDE and anti-BCPDE that, unlike anti-BPDE, are nonplanar and sterically hindered PAH-diol epoxides. The results of the present study indicate that the 1104, A113 variant of hGSTP1-1 is most efficient in the GSH conjugation of both anti-BGCDE and anti-BCPDE. The present study, taken together with previous studies from our laboratory (22, 23) suggest that although V104, V113 variant may play a major role in the detoxification of planar bay-region diol epoxides (e.g., anti-BPDE), the 1104, A113 isoform is likely to be important in the GSH conjugation of nonplanar fjord-region diol epoxides, which are biologically more active than the bay-region diol epoxides (25, 26).

Materials and Methods

Materials. GSH and epoxy-activated Sepharose 6B were purchased from Sigma (St. Louis, MO). The diol epoxides were obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository (Midwest Research Institute, Kansas City, MO).

Expression and Purification of hGSTP1-1 Isoforms. Expression vectors pET9a carrying the cDNAs for the four variants of hGSTP1-1 were prepared as described by us previously (23). The four variants of hGSTP1-1 were purified by GSH-affinity chromatography according to the method of Simons and Vander Jagt (27) with some modifications described by us previously (28).

The purified hGSTP1-1 isoforms were dialyzed against 50 mM Tris-HCl (pH 7.5) containing 2.5 mM KCl and 0.5 mM EDTA (TKE buffer), and stored at −20°C until used. The activity of each variant toward model substrate 1-chloro-2,4-dinitrobenzene was determined by the method of Habig et al. (29) immediately before activity measurements toward diol epoxides.

Determination of GST Activity toward Racemic anti-BGCDE and anti-BCPDE. The GST activity toward anti-BGCDE and anti-BCPDE was determined by the method of Jernström et al. (30) with some modifications. Briefly, the reaction mixture in a final volume of 0.1 ml contained TKE buffer, 2 mM GSH, the desired concentration of the diol epoxide, and 30 μg/ml hGSTP1-1 isoform protein. The reaction was initiated by adding the diol epoxide substrate; the reaction mixture was then incubated for 30 s at 37°C. The GST activity was measured as a function of varying protein concentration and incubation time to optimize the assay conditions. The reaction was terminated by rapid mixing with 0.1 ml cold acetone. Unreacted diol epoxide substrate was removed by extraction with ethyl acetate. The GSH-diol epoxide conjugates in the aqueous phase were quantified by reverse-phase HPLC using a Waters Nova-Pak C18 (3.9 mm × 150 mm) column. The GSH conjugates of (+)- and (-)-anti-BCPDE were eluted isocratically (18.6% acetonitrile in 0.1% trifluoroacetic acid). Under these conditions, the GSH conjugates of (+)- and (-)-anti-BCPDE were eluted at retention times of about 6.6 and 5.8 min, respectively. The separation of GSH conjugates of (+)- and (-)-anti-BGCDE was also achieved by isocratic elution (25.4% acetonitrile in 0.1% trifluoroacetic acid). The retention times of the GSH conjugates of (+)- and (-)-anti-BGCDE were 6.2 and 4.8 min, respectively. The GST activity was measured as a function of varying diol epoxide concentration at a fixed saturating concentration of GSH to determine the kinetic parameters. For anti-BCGDE, the Km and Vmax values were determined by nonlinear regression analysis of the experimental data points using the Michaelis-Menten (hyperbolic) equation as the model. For calculation of the catalytic efficiency, the Michaelis-Menten equation

\[ v = \frac{k_{cat}}{K_m} [E_0] [S] \]

was rearranged to yield:

\[ v = K_m \cdot Q \cdot \frac{[S]}{K_m + [S]} \]

where Q = Vmax/Km. Q is equivalent to the catalytic efficiency (after appropriate change of units for Vmax). Nonlinear regression using the rearranged Michaelis-Menten equation as the model directly yields the catalytic efficiency parameter together with its associated asymptotic SE. The latter constitutes the major advantage of the procedure described above over a simple division of kcat by Km. Although both approaches result in the same value for catalytic efficiency, there is no simple way to arrive at the SE of a ratio of two numbers.

The catalytic efficiencies of hGSTP1-1 variants toward anti-BCPDE were calculated from activity measurements at low substrate concentrations as described by Kilm et al. (31) because saturation curves could not be obtained for this diol epoxide substrate. Poor solubility of anti-BCPDE at higher concentrations was a limiting factor in activity measurements at concentrations of >320 μM.

Results and Discussion

Fig. 1 depicts the reverse-phase HPLC analysis of the water-soluble products resulting from the reaction of 2 mM GSH and 160 μM racemic anti-BGCDE in the presence of 30 μg/ml hGSTP1-1 variant protein. The nonenzymatic GSH conjugation of anti-BGCDE was negligible (data not shown in Fig. 1), whereas this reaction was increased significantly in the presence of hGSTP1-1 protein. All four of the GSTP1-1 variants were virtually inactive toward the (+)-enantiomer of anti-BGCDE. When GST activity was measured as a function of varying anti-BGCDE concentrations (10–160 μM) at a fixed saturating concentration of GSH (2 mM), all four variants of hGSTP1-1 followed Michaelis-Menten kinetics (plots not shown). The kinetic constants for hGSTP1-1 variants toward anti-BGCDE are summarized in Table 1. The catalytic efficiency of hGSTP1-1 (1104, A113) variant was approximately 1.7-fold higher than that of the V104, V113 isomorph (P < 0.05). This was mainly due to a 1.6-fold higher Vmax for the 1104, A113 variant as compared with V104, V113 isomorph. The catalytic efficiencies of the other two variants of hGSTP1-1 toward anti-BGCDE were intermediate but statistically not significantly different from either the 1104, A113 or the V104, V113 isomorph.

As shown in Fig. 2, unlike anti-BGDE, the concentrations of anti-BCGDE approaching saturation of the enzyme could not be attained because of poor solubility of this diol epoxide at higher concentrations. Therefore, a reparameterized Michaelis-Menten rate equation

\[ v = \frac{k_{cat}}{K_m} [E_0] [S] \]

was arranged to:

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SPECIFICITIES OF hGSTPl-1 VARIANTS TOWARD PAH-DIOL EPOXIDES

Table 1  Kinetic constants for hGSTPl-1 variants in the GSH conjugation of anti-BGCD

The activity of each enzyme was measured in triplicate or quadruplicate at five different substrate concentrations. Kinetic parameters were estimated by nonlinear (hyperbolic) regression and are given ± asymptotic error of the mean. The catalytic efficiencies of the 1104,A113 and the V104,V113 variants of the enzyme are significantly different (P < 0.05); the differences in catalytic efficiency between the remaining enzymes do not reach statistical significance.

<table>
<thead>
<tr>
<th>hGSTPl-1 variant</th>
<th>$K_{m}$ (μM)</th>
<th>$V_{max}$ (nmol/min/mg)</th>
<th>$k_{cat}$ (s⁻¹)</th>
<th>Catalytic efficiency (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I104,A113</td>
<td>203 ± 64</td>
<td>95 ± 18</td>
<td>0.074 ± 0.014</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>I104,V113</td>
<td>231 ± 90</td>
<td>85 ± 23</td>
<td>0.066 ± 0.018</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>V104,A113</td>
<td>197 ± 75</td>
<td>78 ± 20</td>
<td>0.060 ± 0.015</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td>V104,V113</td>
<td>223 ± 52</td>
<td>58 ± 20</td>
<td>0.045 ± 0.015</td>
<td>0.21 ± 0.03</td>
</tr>
</tbody>
</table>

Furthermore, we have demonstrated that the catalytic efficiency of the V104,V113 variant is significantly higher compared with I104,A113 isoform in the GSH conjugation of anti-CDE (23), another planar diol epoxide of the bay-region class. These observations led us to postulate that the hGSTPl-1 polymorphism with disparate enzyme activities toward carcinogenic PAH-diol epoxides plays a role in determining overall susceptibility toward certain carcinogens. The results of the present study reveal that hGSTPl-1(I104,A113) variant is most efficient in catalyzing the GSH conjugation of fjord-region diol epoxides. Taken together, our studies suggest that although V104,V113 variant may play an important role in the detoxification of planar bay-region diol epoxides (e.g., anti-BPDE or anti-CDE), the catalytic efficiency ranking is reversed for the nonplanar fjord-region diol epoxides (e.g., anti-BGCD or anti-BCPDE toward which the I104,A113 isoform is most active. Thus, the molecular shape of the diol epoxide substrate seems to be an important determinant for the variable activity of hGSTPl-1 isoforms toward these environmentally relevant carcinogens.

The results of present studies suggest that the putative role of hGSTPl-1 polymorphism in humans with regards to the susceptibility of individuals to PAH-induced carcinogenesis may be more complex than currently realized and should be carefully re-evaluated. It has been shown that hGSTPl-1 polymorphs significantly differ in their catalytic efficiencies in the detoxification of (+)-anti-BPDE (22–24), which suggests differential capabilities of individuals for detoxification of the "ultimate" carcinogenic metabolite of benzo(a)pyrene. However, the frequency of V104,A113 and/or V104,V113 alleles has been shown to be higher in cancer patients compared with I104,A113 allele, which has lower activity toward (+)-anti-BPDE (17, 19, 20, 22, 23).
of the present study, however, clearly demonstrate that the catalytic
differential risk for carcinogenesis.

References
8. Robertson, I. G. C., Jensson, H., Mannervik, B., and Jernström, B. Glutathione S-transferase P1-1(I1104,V1113) allele may have an advantage in the detoxification of the nonplanar PAH-diol epoxides such as anti-BCCDE and anti-BCPDE, which are also abundant among the environmental PAHs (1). In addition, the fjord-region diol epoxides are significantly more mutagenic and carcinogenic in animal models than the diol epoxides of the bay-region class (25, 26). Because PAH mixtures in cigarette smoke, industrial emissions, and other environmental sources are likely to be different, our results indicate that, depending on the chemical composition of the PAH mixture, individuals with a given hGSTPl-l genotype may be at a differential risk for carcinogenesis.
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