Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons

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Introduction

Polycyclic aromatic hydrocarbons (PAH*) represent a widespread class of environmental pollutants, including members that are mutagenic and carcinogenic in experimental animals and, therefore, are highly suspected to play a role as etiological factors in human carcinogenesis (1–4). For instance, cigarette smoking and tumour formation in several organs, in particular the respiratory tract, are intimately associated (5,6). It is likely that exposure to a number of carcinogenic PAHs that occur in cigarette smoke contribute significantly to tumour development in humans (5–7). PAHs require metabolic activation and subsequent formation of adducts with nuclear DNA (3,4,8) to be biologically active, and stereoisomeric bay- and fjord-region diol epoxides, in particular those with (R,S,S,R)-absolute configuration, have been identified as ultimate mutagenic and carcinogenic metabolites of PAH.

Glutathione transferase (GST)-catalysed conjugation of diol epoxides with glutathione (GSH) seems to be of major importance in cellular protection against their genotoxic effects (9–15). Several distinct classes of GSTs exist and each class consists of a varying number of isoenzymes (16). Furthermore, the cellular concentration and distribution of GSTs differ widely among tissues and GST gene expression is regulated by both endogenous and exogenous factors (17). In addition, it has been demonstrated that different GSTs vary significantly in efficiency and enanti selectivity for a number of diol epoxides (12,18–24). Thus, the overall and cell specific capacity of a biological system to resist the harmful activity of diol epoxides is expected to be intimately associated with the nature and the amounts of the GST isoenzymes that are present.

GSTs of the mu and theta classes are genetically polymorphic (for nomenclature of enzymes and GST genes, see Ref. 25). Great interest has been focused on a possible correlation between lack of these isoenzymes and increased susceptibility of tumour formation. Although not entirely conclusive, several studies suggest that such a correlation exists (26,27).

The GSTP1 gene has been shown to exist in allelic variants (28–31), and recent epidemiological studies indicate that individuals with the GSTP1* B allele encoding the GSTP1-1 protein with valine (GSTP1-1/V-105) rather than isoleucine at position 105 (GSTP1-1/I-105)¹, may be more susceptible to benign and malignant disease because of other factors than a decreased catalytic efficiency of GSTP1-1/V-105 in the detoxication of carcinogenic diol epoxides of benzo[a]pyrene or structurally related PAH.

Previous studies have identified allelic variants of the human glutathione transferase (GST) Pi gene and showed that the two different encoded proteins with isoleucine (GSTP1-1/I-105) or valine (GSTP1-1/V-105) at position 105, respectively, differ significantly in their catalytic activities with model substrates. Moreover, recent epidemiological studies have demonstrated that individuals differing in the expression of these allelic variants also differ in susceptibility to tumour formation in certain organs, including such in which polycyclic aromatic hydrocarbons (PAH) may be etiological factors. In the present study the catalytic efficiencies (k_cat/K_m) of these GSTP1-1 variants were determined with a number of stereoisomeric bay-region diol epoxides, known as the ultimate mutagenic and carcinogenic metabolites of PAH, including those from chrysene, benzo[a]pyrene and dibenz[a,j]anthracene. In addition, GSTP1-1 mutants in which amino residue 105 is alanine (GSTP1-1/A-105) or tryptophan (GSTP1-1/W-105) have been constructed and characterized. GSTP1-1/V-105 was found to be more active than GSTP1-1/I-105 in conjugation reactions with the bulky diol epoxides of PAH, being up to 3-fold as active towards the anti- and syn-diol epoxide enantiomers with R-absolute configuration at the benzylic oxiranyl carbon. Comparing the four enzyme variants, GSTP1-1/A-105 generally demonstrated the highest k_cat/K_m value and GSTP1-1/W-105 the lowest with the anti-diol epoxides. A close correlation was observed between the volume occupied by the amino acid residue at position 105 and the value of k_cat/K_m. With the syn-diol epoxides, such a correlation was observed with alanine, valine and isoleucine, whereas tryptophan was associated with increased k_cat/K_m values. The mutational replacement of isoleucine with alanine or tryptophan at position 105 did not alter the enantio selectivity of the GSTP1-1 variants compared with the naturally occurring allelic variants GSTP1-1/I-105 and GSTP1-1/V-105. Since the amino acid at position 105 forms part of the substrate binding site (H-site) the effect of increasing bulkiness is expected to cause restricted access of the diol epoxide and proper alignment of the two reactants for efficient glutathionyl-

¹The numbering of amino acid residues includes the initiation methionine as number 1 in agreement with the numbering of codons (cf. ref. 16); the site of allelic variations is thus at residue 105. Some publications refer to this position as 104.

*Abbreviations: PAH, polycyclic aromatic hydrocarbons; CDNB, 1-chloro 2,4-dinitrobenzene; syn- and anti-CDE, syn- and anti-chrysene-1,2-diol 3,4-epoxide; anti-BPDE, anti-benzo[a]pyrene-7,8-diol 9,10-epoxide; syn- and anti-DBADE, syn- and anti-dibenz[a,j]anthracene-1,2-diol 3,4-epoxide.
susceptible to tumour formation in organs exposed to PAH (32–34).

In this study, the activity has been determined of the naturally occurring GSTP1-1/I-105 and GSTP1-1/V-105 variants towards a series of selected stereoisomeric bay-region diol epoxide substrates of carcinogenic PAHs, in order to provide a possible explanation for differences in cancer susceptibility in populations varying in the distribution of this important class of phase II enzymes. In addition, the effect of the size of amino acid residue 105 on the efficiency of GSTP1-1 to catalyse the conjugation of diol epoxides with GSH has been investigated by examination of variants with alanine (GSTP1-1/A-105) and tryptophan (GSTP1-1/W-105), respectively, at this position.

Materials and methods

Chemicals
The syn- and anti-diol epoxide enantiomers of chrysene (syn- and anti-CDE), benzo[a]pyrene (syn- and anti-BPDE) and dibenz(a,h)anthracene (syn- and anti-DBADE) were synthesized as reported previously (21). The purity of each individual stereoisomer was >95% as determined by HPLC. Chromatographic standards of glutathione conjugates of diol epoxides were obtained as described in earlier studies (20,21).

Glutathione transferases

The GSTP1-1 variants were obtained by heterologous expression in Escherichia coli (35) and purified by a modified affinity chromatography method (36). The purified enzyme (5–10 mg/ml dissolved in 10 mM Tris–HCl, 10 mM dithiothreitol and 0.02% sodium azide, pH 7.8) was used freshly prepared or after storage at 4°C. Prior to use, the required amount of enzyme was freed of dithiothreitol and sodium azide by passage through a NAP-10 column (Pharmacia, Uppsala, Sweden) previously washed and equilibrated with 50 mM Tris–HCl, pH 7.5. Prior to each experiment, the enzymic activity was estimated with CDNB (37). The following specific activities of the GSTP1-1 variants were used to calculate the amount of active proteins: I-105: 98, V-105: 59, A-105: 37 and W-105: 56 µmol CDNB/mg per min.

Incubations

Enzyme corresponding to 50–500 µg active protein/ml was incubated at 37°C for 30 s or 1 min with 40 and 80 µM diol epoxide (added in dimethyl sulfoxide, final concentration 5%; v/v) and 5 mM GSH in 50 mM Tris–HCl-buffer, pH 7.5 (final volume 100 µl) and analysed for GSH conjugates by HPLC as described (20,21).

Results and discussion

Recent epidemiological studies have indicated that GSTP1-1 allelic polymorphism may be an important factor in cancer susceptibility in organs exposed to PAH (32–34). The results demonstrated that populations with a high frequency of the GSTP1* B allele (coding for GSTP1-1/Val-105) might be at higher risk for tumour formation by PAH than those with the GSTP1* A allele (coding for GSTP1-1/Ile-105). Therefore we considered it to be of importance to determine the catalytic efficiencies (kcat/Km) of GSTP1-1 variants towards a set of stereoisomeric bay-region diol epoxides of several carcinogenic PAHs (see Figure 1 for the compound employed and their structures).

The kcat/Km values obtained are compiled in Table I. The results obtained with GSTP1-1/V-105 and GSTP1-1/I-105 show that replacing valine by the bulkier isoleucine reduces the kcat/Km value ~3-fold for the (+)-anti-enantiomers of CDE and BPDE and the (+)-syn-enantiomer of DBADE. No effect on the kcat/Km value was observed with (+)-syn-CDE, whereas with (+)-syn-BPDE and (+)-anti-DBADE a slight reduction was observed. The incidence of lung, testis and bladder cancer has been reported to be increased in individuals expressing

![Figure 1](image-url)
Glutathione conjugation of polycyclic aromatic hydrocarbon diol epoxides

Table I. Catalytic efficiency ($k_{cat}/K_m$) for human GSTP1-1 variants with bay region diol epoxides of PAH

<table>
<thead>
<tr>
<th>Substrate</th>
<th>GSTP1-1/A-105</th>
<th>GSTP1-1/V-105</th>
<th>GSTP1-1/I-105</th>
<th>GSTP1-1/W-105</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-anti-CDE</td>
<td>65 ± 11</td>
<td>9.9 ± 1.8</td>
<td>3.6 ± 1.0</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>(+)-syn-CDE</td>
<td>105 ± 21</td>
<td>11 ± 1.0</td>
<td>11 ± 3.2</td>
<td>20 ± 2.8</td>
</tr>
<tr>
<td>(+)-anti-BPDE</td>
<td>92 ± 8.3</td>
<td>20 ± 2.2</td>
<td>7.5 ± 1.9</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>(+)-syn-BPDE</td>
<td>190 ± 28</td>
<td>100 ± 11</td>
<td>64 ± 0.3</td>
<td>190 ± 10</td>
</tr>
<tr>
<td>(+)-anti-DBADE</td>
<td>9.8 ± 1.1</td>
<td>1.9 ± 0.7</td>
<td>1.4 ± 0.4</td>
<td>0.4 ± 0.03</td>
</tr>
<tr>
<td>(+)-syn-DBADE</td>
<td>16.5 ± 1.3</td>
<td>5.4 ± 0.7</td>
<td>2.0 ± 0.6</td>
<td>34 ± 3.3</td>
</tr>
</tbody>
</table>

*Volume (Å³) of amino acid residue, alanine 69; valine 120; isoleucine 204; tryptophan 245.

References


105 in human GSTP1-1 and its effect on the catalytic activity towards (+)-anti-BPDE. Consistent with our findings, they observed that GSTP1-1/V-105 was more active than GSTP1-1/I-105 in S-glutathionylation of the diol epoxide. Based on molecular modelling, a mechanism was proposed to explain the difference in activity. It is known that the H-site of GSTP1-1 is partially hydrophobic and partially hydrophilic. The hydrophilic character is due to arginine and asparagine in conjunction with hydrogen-bonded water molecules (40). It was suggested that binding of the (+)enantiomer of anti-BPDE in the H-site of GSTP1-1/I-105 allows a favourable interaction between polar constituents of the H-site and the hydroxyl groups of the diol epoxide, in addition to the interaction of the pyrenyl residue of BPDE with the hydrophobic part (phenylalanine, valine and glycine). It was suggested that substituting valine with the bulkier isoleucine severely disturbs the hydrophilic character of the H-site, and consequently results in a less efficient interaction with (+)-anti-BPDE and lower activity (39).

Recent molecular modelling studies in our laboratory demonstrated that the enantiomers of anti- and syn-BPDE with R-absolute configuration at the benzylic oxirane carbon could be snugly fitted in the H-site of GSTP1-1/I-105 and close to the GSH sulphur, whereas those with opposite stereochemistry could not (38). Moreover, the tyrosine at position 109 may well contribute to the favourable fit $\pi-\pi$ interaction with the pyrenyl residue of the diol epoxide. In addition, the localization of Y-109 suggested its participation in catalysis by hydrogen-bonding of the hydroxyl group to the arene oxide and thus facilitating ring opening. This suggestion is supported by a recent study by Ji et al. (40).

Taken together, the molecular modelling studies seem to be compatible with the functional studies with both anti and syn-diol epoxides. However, the lowered activity observed with increasing size of the amino acid residue 105 (Ala→Ile→Val→Trp) and the anti-diol epoxides is in addition to a possible disturbance of the hydrophilic character of the H-site, also probably a consequence of a restricted H-site. This in turn may limit free access of the substrate and proper alignment of the diol epoxide, and the thiolate form of glutathione, to allow efficient conjugation. The enhancement of the activities towards the syn-diol epoxides caused by the substitution of tryptophan is unclear.

Extended functional studies are needed in conjunction with additional molecular modelling to further elucidate mechanistically the influence of amino acid replacement at specific positions contributing to the H-site structure and consequent changes in catalytic efficiency and substrate selectivity.

In conclusion, the elevated activity of the naturally occurring GSTP1-1/V-105 in comparison with GSTP1-1/I-105 towards bay-region diol epoxides with ($R,S,S,R$)-absolute configuration indicates that the correlation between the GSTP1-1B allele and the suggested higher risk for PAH carcinogenesis is not caused by a lower catalytic efficiency of the corresponding enzyme. This may have a number of alternative explanations, one possibility is that the diol epoxide derivatives studied here are not etiological factors in the organs believed to be susceptible to PAH exposure. It is also possible that a concomitant polymorphism in the metabolically competent and PAH-activating cytochrome P450 isoforms may play a dominant role for the cancer susceptibility towards PAH. However, since the present study has been restricted to bay-region diol epoxides of a few selected PAH, the possibility remains that the important tumour initiators are among the more potent fjord-region diol epoxides (41–44), which may behave differently in the conjugation reactions catalyzed by the GSTP1-1 variants. This possibility is presently under investigation in our laboratory. Finally, it has been noted that GSTP1-1/V-105 is significantly less thermo-stable than GSTP1-1/I-105 (A.-S. Johansson, G. Stenberg, M. Widersten and B. Mannervik, unpublished work), which may provide an explanation for the over-representation of the allelic variant GSTP-B in patients with certain types of tumour.

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