Metabolic activation of methyl-hydroxylated derivatives of 7,12-dimethylbenz[a]anthracene by human liver dehydroepiandrosterone-steroid sulfotransferase

H.-C. Chou, S. Ozawa, P. P. Fu, N. P. Lang and F. F. Kadlubar

Introduction

Polycyclic aromatic hydrocarbons (PAHs*) are widespread genotoxic environmental pollutants (1–3). PAHs, both unsubstituted and alkyl-substituted, are generated from incomplete combustion of organic matters (1). Thus, they are present in fossil fuels, motor vehicle wastes, residential furnaces, natural gas combustion, char-broiled foods, tobacco smoke and many other sources (1,2,4). A variety of methyl-substituted PAHs has been found in environmental samples and many of them have been determined to be potent carcinogens (3,4). Among the carcinogenic methyl-substituted PAHs, the metabolic activation of 7,12-dimethylbenz[a]anthracene (DMBA), 5-methylchrysene, 7-methylbenz[a]anthracene, 3-methylcholanthrene and 6-methylbenz[a]pyrene have been most extensively studied (3–6).

The formation of strongly electrophilic bay-region (7,8) diol epoxide metabolites of several PAHs has been generally regarded as a major metabolic pathway that leads to DNA adduct formation and carcinogenic activity. The tumors induced by these PAHs are, however, dependent upon their mode of administration and other experimental conditions (9–11). The complexity of PAH and methylated PAH carcinogenesis thus implies multiple mechanisms of activation arising from different reactive ultimate metabolites (12–14). The predominance of one mechanism over another may change, depending on the nature of the activating enzymes present in the target organ of different species. It has been postulated that other reactive electrophilic intermediates, such as radical cations (15,16) and benzyl carbencium ions of alkylated PAHs generated via formation of benzyl esters (17,18), might also be ultimate carcinogenic forms of PAHs.

Hydroxylation of the alkyl side chain in most tissues represents a major pathway in the metabolism of alkyl-substituted PAHs (9,12–14,19–22). Subsequent formation of the electrophilic sulfuric acid esters has been found (23–27) in studies on rodent sulfotransferase-catalyzed activation of several hydroxymethyl-PAHs. These results indicated that the bioactivation of hydroxymethyl-PAHs by rodent tissue cytosols is catalyzed by dehydroepiandrosterone (DHEA)-steroid sulfotransferase, but not by phenol sulfotransferases (28).

In human liver, five isoforms of cytosolic sulfotransferase have thus far been found to be expressed: DHEA-steroid sulfotransferase, two thermostable (TS)- and one thermolabile (TL)-phenol sulfotransferase, and an estrogen sulfotransferase. These results indicate that the sulfotransferase-mediated activation of the methylhydroxylated DMBAs is predominantly catalyzed by DHEA-steroid sulfotransferase in human liver and that TS- and TL-phenol sulfotransferases and estrogen sulfotransferase are not involved in the catalysis.

*Abbreviations: PAHs, polycyclic aromatic hydrocarbons; DMBa, 7,12-dimethylbenz[a]anthracene (DMBA); DHEA, dehydroepiandrosterone; TS, thermostable; TL, thermolabile; 7-OH-DMBA, 7-hydroxymethyl-12-methylbenz[a]anthracene; 12-OH-DMBA, 12-hydroxymethylbenz[a]anthracene; 7,12-diOH-DMBA, 7,12-dihydroxymethylbenz[a]anthracene; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; DCNP, 2,6-dichloro-4-nitrophenol.
PAHs to form reactive intermediate(s) capable of binding to DNA in cytosolic fractions from several human tissues. In order to determine the role of the different human tissue sulfotransferases mentioned above, we examined the effects of substrates and inhibitors, that are specific to the DHEA-steroid, phenol and estrogen sulfotransferases, on the metabolic activation of 7-OH-DMBA, 7-methyl-12-hydroxymethylbenza[α]anthracene (12-OH-DMBA) and 7,12-dihydroxymethylbenza[α]anthracene (7,12-diOH-DMBA).

Materials and methods

Chemicals
Tritiated 7-OH-DMBA (49 mCi/mmol), 12-OH-DMBA (127 mCi/mmol) and 7,12-diOH-DMBA (100 mCi/mmol) were synthesized by oxidation of the methyl-hydroxylated DMBA's to their corresponding formal derivatives followed by reduction with sodium borotritide, which resulted in tritium specifically bound to benzylic carbons (38). Purity was judged to be >98% by high pressure liquid chromatography (38).

1-Phosphoadenosine-5'-phosphosulfate (PAPS), dopamine, p-nitrophenol, p-nitrophenyl sulfate, DHEA, estrone, and calf thymus DNA (type I) were purchased from the Sigma Chemical Co. (St Louis, MO). [35S]PAPS (2.5 Ci/mmol) and [1,2-3H(H)]DHEA (55.6 mCi/mmol) were obtained from DuPont-NEN Research Products (Boston, MA). 2,6-Dichloro-4-nitrophenol (DCNP) was from ICN Pharmaceuticals, Inc. (Costa Mesa, CA).

Tissue and cytosol preparation
Human tissues were obtained as surgical samples from the John L.McClellan Memorial Veterans’ Hospital and the US Cooperative Tissue Network. These tissues were excess surgical samples that were immediately frozen in liquid N2 and stored at -80°C before use. Cytosols were prepared in 10 mM triethanolamine buffer (pH 7.4) containing 0.25 M sucrose and 5 mM 2-mercaptoethanol using a Polytron homogenizer as previously described (39).

Protein concentrations were determined by the biuret reaction (40).

Sulfotransferase assays
Human cytosol-mediated sulfation of the methyl-hydroxylated derivatives of DMBA was carried out at 37°C under argon saturation using the conditions described previously (39). Briefly, the reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.5), 5 mM MgCl2, 0.5 mM EDTA, calf thymus DNA (2 mg/ml), 0.2 mM PAPS, cytosol protein and radiolabeled substrates: 7-OH-DMBA (20 µM), 12-OH-DMBA (10 µM) or 7,12-dioH-DMBA (100 µM). The reactions were carried out at 37°C for 20 min and enzyme activity was measured as the PAPS-dependent binding of the radiolabelled methyl-hydroxylated DMBA derivatives to DNA (39).

TS-phenol sulfotransferase activity was measured by two different methods: (i) the sulfation of p-nitrophenol (4 µM) with [35S]PAPS as cofactor (41); and (ii) the sulfation of 2-naphthol (0.1 mM) with a PAPS-regenerating system (using p-nitrophenylsulfate), where enzyme activity was determined by substrate (2-naphthol)-dependent release of p-nitrophenol (41). Briefly, the reaction mixture (0.5 ml) contained 50 mM potassium phosphate buffer (pH 6.3), 5 mM MgCl2, 20 µM PAPS, 5 mM p-nitrophenylsulfate and human tissue cytosols. After a 10 min incubation at 37°C, the reaction was terminated by addition of an equal volume of 0.25 M Tris–HCl buffer (pH 8.7) and 6 vol of water-saturated chloroform. The concentration of p-nitrophenol was then determined in the aqueous phase (ε305 nm = 18 200/M). TL-phenol sulfotransferase, DHEA-ST and estrogen sulfotransferase assays were conducted at 37°C for 10 min as previously described (39,41,42) with 10 µM dopamine, 3 µM DHEA and 20 nM estrone as substrates, respectively.

Under these conditions, the rates of sulfation were linear with time, first order with respect to cytosol protein concentration (0.5–3.0 mg/ml), and showed substrate saturation for the methyl-hydroxylated DMBA derivatives. The concentrations of p-nitrophenol and 2-naphthol, dopamine, DHEA and estrone were chosen to reflect specific catalysis and substrate saturation (39,41–44) for the major TS-phenol sulfotransferase, TL-phenol sulfotransferase, DHEA-steroid sulfotransferase and for estrogen sulfotransferase, respectively.

Results

Methyl-hydroxylated DMBA sulfotransferase activities: Inhibitory effects of DHEA and DCNP
All three of the methyl-hydroxylated derivatives of DMBA studied were metabolically activated by human liver cytosolic sulfotransferase to form reactive metabolites that covalently bind with DNA. With various concentrations of DHEA, a high affinity substrate for human DHEA-steroid sulfotransferase (29,30), added to the reaction mixture, the PAPS-dependent covalent DNA binding of all three DMBA derivatives was strongly inhibited in a dose-related fashion (Figure 1). At 10 µM DHEA, the PAPS-dependent DNA binding of 7-OH-DMBA, 12-OH-DMBA and 7,12-dioH-DMBA was inhibited by 73, 65 and 62%, respectively.

DCNP, which is widely used as a potent inhibitor of TS-phenol sulfotransferases (45,46), has recently been shown to inhibit strongly DHEA and estrone sulfotransferase, albeit at higher DCNP concentrations of 30–100 µM (46,47). Sulfation of neither the three methyl-hydroxylated DMBA substrates nor DHEA was significantly affected by 2–20 µM DCNP, while p-nitrophenol and 2-naphthol sulfotransferase activities were almost completely suppressed (>95%) over this concentration range (data not shown).

The effect of substrates for phenol and estrogen sulfotransferases
Three distinct enzymes having phenol sulfotransferase activity in humans have been described based on a number of different properties, including substrate specificity, chromatographic separation, inhibitor sensitivity and thermal stability (43–48). Two TS-phenol sulfotransferase isoforms sulfate simple neutral phenols such as 2-naphthol and p-nitrophenol, whereas TL-phenol sulfotransferase preferentially catalyzes the sulfate conjugation of phenolic monoamines such as dopamine and epinephrine. p-Nitrophenol is also a high affinity substrate for estrogen sulfotransferase (42). Accordingly, the effect of the typical substrates for TS- and TL-phenol and estrogen sulfotransferases on 12-OH-DMBA sulfation by human liver cytosol was examined. No significant inhibitory effects on PAPS-dependent DNA binding of 12-OH-DMBA were observed in the presence of either p-nitrophenol and dopamine (1–100 µM) or of estrone (1 µM) (data not shown).
Activation of 7,12-dimethylbenz[a]anthracene

Fig. 2. Individual correlation of the PAPS-dependent DNA binding of 12-OH-DMBA with sulfotransferase activities toward p-nitrophenol, 2-naphthol, dopamine and DHEA in human liver cytosols (n = 12). PAPS-dependent DNA binding of [3H]12-OH-DMBA (pmol bound/mg DNA) was measured as described in Materials and methods. Each point is the average of two determinations; the values obtained were within 5% of each other. Statistical analyses were conducted using the Spearman rank order correlation test.

Individual correlation studies

Twelve human liver cytosols were prepared to investigate inter-individual variation and to correlate sulfotransferase activities for 12-OH- and 7,12-diOH-DMBA to those toward 2-naphthol and p-nitrophenol (substrates for TS-phenol and estrogen sulfotransferases), dopamine (a TL-phenol sulfotransferase substrate) and DHEA. PAPS-dependent DNA binding of 12-OH- (Figure 2) and 7,12-diOH-DMBA (Figure 3), correlated well with DHEA-ST activities with correlation coefficients of r = 0.90 and 0.92, respectively. Although there was a weak correlation between TL-phenol and 12-OH-DMBA sulfotransferase activities (r = 0.70), there was no significant correlation with 7,12-diOH-DMBA activity. Moreover, there was no correlation with sulfotransferase activities measured using either the 35S-PAPS assay with p-nitrophenol or the colorimetric assay with 2-naphthol. A comparison of DHEA-steroid and estrogen sulfotransferase activities in another 26 human liver cytosols likewise showed no apparent correlation (data not shown).

Tissue distribution

Additional evidence for these methyl-hydroxylated DMBA derivatives being specific substrates for human DHEA-steroid sulfotransferase was obtained through the examination of the tissue distribution for the metabolic activation of the three hydroxymethyl derivatives of DMBA. Among cytosols prepared from human liver, colon, pancreas, larynx and breast, DHEA-steroid sulfotransferase and the PAPS-dependent metabolic activation of the methyl-hydroxylated DMBA derivatives were found only with human liver, while both TS- and TL-phenol sulfotransferases were notably active in both human liver and colon cytosols (Table I).

Discussion

In the present study, we have shown that 7-OH-DMBA, 12-OH-DMBA and 7,12-diOH-DMBA undergo enzymatic PAPS-dependent metabolic activation in human liver cytosols to form reactive intermediates capable of covalent binding to DNA. We also present evidence from substrate and inhibition
Fig. 3. Individual correlation of the PAPS-dependent DNA binding of 7,12-diOH-DMBA with sulfotransferase activities toward \( p \)-nitrophenol, 2-naphthol, dopamine and DHEA in human liver cytosols (\( n = 12 \)). PAPS-dependent DNA binding of \( [3H]7,12\text{-diOH-DMBA} \) (pmol bound/mg DNA) was measured as described in Materials and methods. Each point is the average of two determinations; the values obtained were within 5% of each other. Statistical analyses were conducted using the Spearman rank order correlation test.

### Table I. Tissue distribution of sulfotransferase activity toward \( p \)-nitrophenol, dopamine, DHEA and three methyl-hydroxylated derivatives of DMBA

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Liver</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Nitrophenol</td>
<td>519.5</td>
<td>190.0</td>
</tr>
<tr>
<td>Dopamine</td>
<td>93.5</td>
<td>462.3</td>
</tr>
<tr>
<td>DHEA</td>
<td>137.7</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>7-OH-DMBA</td>
<td>21.4</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>12-OH-DMBA</td>
<td>13.1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>7,12-diOH-DMBA</td>
<td>15.2</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

Sulfotransferase activity for these substrates was determined in human liver cytosol (3 mg/ml; 20 min) as described in Materials and methods, and the rates are expressed as pmol substrate metabolized/min per mg protein for \( p \)-nitrophenol, dopamine and DHEA and as pmol \( ^3 \text{H} \)-substrate bound/mg DNA for 7-, 12- and 7,12-diOH-DMBA.

studies, correlational analyses and tissue distribution data that human DHEA-steroid sulfotransferase is primarily responsible for the bioactivation of these methyl-hydroxylated DMBA substrates. Furthermore, these data are consistent with the observations by Glatt and co-workers that cDNA-expressed human DHEA-steroid sulfotransferase markedly potentiates 7-OH-DMBA mutagenicity in *Salmonella typhimurium* (37). These data are in clear contrast to our previous studies on the \( N \)-hydroxy derivatives of carcinogenic aromatic and heterocyclic amines, which were activated by human liver TS-phenol sulfotransferases to form reactive sulfuric acid esters; neither TL-phenol sulfotransferase nor DHEA-steroid sulfotransferase showed a significant contribution to the activation of these compounds (31,39,41).

In extensive studies of rodent carcinogenesis, DMBA and its methyl-hydroxylated metabolites have been shown to induce hepatomas in male B6C3F1 mice (21), lung adenomas in CD-1 mice (10), mouse skin tumors (49), preneoplastic foci in rat livers (21) and rat mammary gland tumors (50). This wide variety of target organs implies that diverse metabolic activation pathways are required for the multiple target tissue carcinogenicity induced by PAHs. In the present study, an appreciable capacity for the activation of 7-OH-, 12-OH- and 7,12-diOH-DMBA was detected only in livers of the human tissues studied. However, among the possible human target tissues for PAHs, cytosols from human lung showed low but detectable DHEA-steroid sulfotransferase activity (51–53). No appreciable DHEA-steroid sulfotransferase was detected in normal breast...
tissues by immunostaining, whereas the enzyme was detected in breast tumor tissues and breast cancer cell lines (54). Recently, estrogen was shown to potentiate the human DHEA-steroid sulfotransferase gene promoter (55). Thus, alteration of the activation capacity of methyl-hydroxylated PAHs resulting from regulation of DHEA-steroid sulfotransferase may become an important bioactivation pathway in target tissues regulated by endocrine factors.

It has been suggested that different sulfotransferase gene families show overlapping substrate specificities (56). Human DHEA-steroid sulfotransferase is 37% identical and 60% similar to the sequence of human estrogen sulfotransferase (30). Indeed, DHEA-steroid sulfotransferase showed the optimal activity at 3 µM DHEA, whereas estrogen sulfotransferase exhibited the optimal activity at a DHEA concentration of 30 µM (30,42,48). Conversely, optimal 17β-estradiol and estrone sulfotransferase activities were shown to be optimal at a 20 nM substrate concentration for estrogen sulfotransferase, whereas DHEA-steroid sulfotransferase activity required 1000-fold higher concentration of estradiol and estrone than did estrogen sulfotransferase (30,42,48). We observed that the activating capacities of 12-OH- and 7,12-diOH-DMBA were highly correlated with DHEA-steroid sulfotransferase activity in human livers. Moreover, DHEA-steroid sulfotransferase activity did not correlate with estrogen sulfotransferase activity in human livers, which was similar to the observation recently reported by Her et al. with human small intestine (57). These results further supported the conclusion that only liver DHEA-steroid sulfotransferase primarily contributes to the sulfotransferase-mediated metabolic activation of 7-OH-, 12-OH- and 7,12-diOH-DMBA.

Of potential significance to in utero carcinogenesis is the high expression of DHEA-steroid sulfotransferase activity in human fetal tissues. DHEA-sulfate is secreted from the fetal adrenal into circulation during fetal development. Subsequently, the sulfate group is removed by sulfatase activity in fetal livers, which was similar to the observation recently reported by Her et al. with human small intestine (57). These results also raise the possibility that maternal exposure to methylated and/or methyl-hydroxylated PAHs could result in transplacental carcinogenesis.

References


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