Metabolism of carcinogenic heterocyclic and aromatic amines by recombinant human cytochrome P450 enzymes

George J. Hammons, Daria Milton, Kristy Stepps, F. Peter Guengerich, Robert H. Tukey and Fred F. Kadlubar

National Center for Toxicology Research, Jefferson, AR 72079, 1Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt School of Medicine, Nashville, TN 37232 and 2Cancer Center, University of California at San Diego, La Jolla, CA 92039, USA

The N-hydroxylation of carcinogenic arylamines represents an initial step in their metabolic activation. Animal studies have shown that this reaction is catalyzed by the cytochrome P450 (P450) enzymes P450 1A1 and P450 1A2. In this study, utilizing enzymes expressed in Escherichia coli (and purified) or in human B-lymphoblastoid cells, the catalytic activities of recombinant human P450 1A1, P450 1A2, and P450 3A4 for N-hydroxylation of several carcinogenic arylamines were determined. P450 1A2 from both expression systems catalyzed the N-hydroxylation of 4-aminobiphenyl and the heterocyclic amines, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Rates were similar, with values of 1.1–7.8 nmol/min/nmol P450. In contrast, P450 1A1 catalyzed N-hydroxylation of only PhIP, and no activity was observed with P450 3A4. Further kinetic analysis with purified P450 1A2 showed similar K_m and V_max values for N-hydroxylation of the arylamines. Furafylline and fluvoxamine, inhibitors of P450 1A2 activity in human liver microsomes, were found to be inhibitory of the recombinant P450 1A2 N-hydroxylation activity. Results from this study are supportive of a major role for human P450 1A2 in the metabolic activation of arylamines.

The activation and detoxification of chemical carcinogens by cytochrome P450 (P450*) enzymes have been extensively studied (1,2). There is considerable evidence that variations in the levels of P450s in experimental animals can influence tumor formation since the relative rates of metabolic activation and/or detoxification of carcinogens can be critical determinants in tumor induction (1,3). Levels of expression of different human P450 enzymes differ considerably among individuals, and there has been much interest in evaluating the influence of these differences in risk to potential carcinogens (1,3–5). As a means of understanding the relationship between P450s and cancer in humans, the roles of individual P450 enzymes in the metabolism of carcinogens need to be identified.

The importance of aromatic amines, including heterocyclic amines, in the etiology of human cancer is of growing interest (6). Most heterocyclic amines formed during the normal cooking of meat products have been shown to be strong mutagens (7,8). Three heterocyclic amines in this group are 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). PhIP and IQ have also been found in mainstream cigarette smoke (9) and in beer and wine (10). IQ, MeIQx, and PhIP are potent multi-organ carcinogens in rodents (11–14). PhIP has been shown to induce mammary and colon adenocarcinomas in F344 rats when administered in diet. IQ and MeIQx cause tumors in liver, lung, Zymbal’s gland, and other sites in F344 rats. IQ has also been shown to be a hepatocarcinogen in non-human primates (15).

Heterocyclic and aromatic amines, as many other chemical carcinogens, require metabolic activation to convert them to genotoxic products. The in vitro metabolism and activation of these chemicals have been studied in several animal species, both with microsomal preparations and purified enzymes in reconstituted systems (16–21). These studies have shown that the initial step in the activation is N-hydroxylation and the P450 enzymes catalyze the reaction. Metabolic activation and N-hydroxylation of heterocyclic amines have also been demonstrated with human liver microsomes (21–26). Involvement of P450 1A2 has been implicated by correlation of activity with P450 1A2 content or by inhibition with specific antibodies or a selective chemical inhibitor. Further evidence has been provided by studies in which recombinant human P450 1A2 has been shown to catalyze the mutagenic activation of IQ, MeIQx, PhIP, and other heterocyclic amines (23,27,28). However, only one study has provided direct demonstration of purified human P450 1A2 catalyzing the N-hydroxylation of an arylamine (29). In the present study, we have utilized recombinant human P450 enzymes to establish directly the role of individual P450s in the N-hydroxylation of these carcinogenic arylamines and to provide support for this activation pathway in humans.

Radiolabeled ABP (2,2'3H; 32 mCi/mmol), IQ (ring-3H; 74 mCi/mmol), and PhIP (ring-3H; 13.5 Ci/mmoll were obtained from ChemSyn Science Laboratories (Lenexa, KS). [2–14C]MeIQx was acquired from Toronto Research Chemicals (Ontario, Canada). Furafylline was purchased from Ultrafine Chemicals (Manchester, England), and fluvoxamine was kindly donated by Solvay DuPhar (Weesp, The Netherlands). Other chemicals were commercially obtained and of HPLC or analytical grade. Human P450 1A1 (30), P450 1A2 (31), and P450 3A4 (32,33) were expressed in Escherichia coli, purified, and constituted as previously described. cDNA recombinant human P450 1A1 (M111b, lot 6) and P450 1A2 (M103c, lot 15) were purchased from Gentest (Woburn, MA). These enzymes were expressed in B-lymphoblastoid cells and obtained as microsomal preparations. A microsomal preparation from control cells (M101a, lot 16) was also purchased. NADPH-cytochrome c (P450) reductase was prepared as described by Yasukochi and Masters (34) with slight modification (35). Cytochrome b5 was purified as described elsewhere.

*Abbreviations: P450, cytochrome P450; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; ABP, 4-aminobiphenyl.
microsomes from human liver have also been determined to be inhibitory to P450 1A2 activity in humans, the inhibition experiments, furafylline or fluvoxamine was added to the assays has also been shown to be limited (27, 40). Metabolic assays were conducted with ABP, IQ, MeIQx, or PhIP and microsomal preparations of human P450 enzymes which were expressed in E. coli. At a substrate concentration of 0.1 mM, P450 1A2 from both expression systems was found to catalyze the N-hydroxylation of each of the arylamines at significant rates. Rates of N-hydroxylation of ABP and the heterocyclic amines with enzyme expressed in either system were similar (Table I). With P450 1A2 expressed in E. coli, the values ranged from 1.1 (± 0.1) nmol/min/nmol P450 to 3.8 (± 0.5) nmol/min/nmol P450. The range with enzyme expressed in B lymphoblastoid cells was 2.0 (± 0.1) nmol/min/nmol P450 to 7.8 (± 1.0) nmol/min/nmol P450. Interestingly, the rate of N-oxidation of ABP (1.4 nmol/min/nmol P450) catalyzed by P450 1A2 purified from human liver was similar in value (29). Similarity in the rates of N-oxidation of these arylamine substrates was also found when assays were conducted with human hepatic microsomes from the same individual (22). Rates of N-oxidation of PhIP with microsomes from human liver have also been determined in at least two other studies (26, 37). These values were somewhat lower but a wide interindividual variation in N-hydroxy-PhIP formation among humans was observed, presumably reflecting varying levels of P450 1A2 in the liver among individuals. Recombinant human P450 1A2 has also been shown to catalyze the activation of heterocyclic amines in mutagenic assays (23, 27). The level of activation was similar for IQ and MeIQx; it was less for PhIP.

In contrast to results observed with P450 1A2, the 1A1 enzyme was not found to catalyze the N-hydroxylation of the arylamines included in this study (Table I). The one exception was the N-hydroxylation of PhIP with enzyme expressed in E. coli; however, the rate was much lower than that observed with P450 1A2. Our results are consistent with those found when these two enzymes were compared for their ability to catalyze the activation of heterocyclic amines to mutagens (23, 27). Recombinant human P450 1A1 was a much less effective catalyst for IQ and MeIQx than P450 1A2; with PhIP its efficiency is reported to be lower but only 50% less active. The human enzyme may differ with that of rat or rabbit at least with respect to the substrate PhIP. Purified rat liver P450 1A1 was found to catalyze the N-hydroxylation of PhIP at a rate similar to that of purified P450 1A2 (17), and the purified enzyme from rabbit was a more effective catalyst in activating this substrate than the purified 1A2 enzyme (18). However, with 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) purified rabbit P450 1A1 was reported to be 25 times less active than P450 1A2 in converting this substrate to a mutagen in the Ames test (38). The ability of P450 3A4 to catalyze the N-hydroxylation of aryldamines was also examined in the present study. This enzyme is the most abundant P450 in human liver (39). N-Hydroxylation activity by P450 3A4 was not observed with any of the arylamines examined (Table I). The ability of this enzyme to activate several of these amines in mutagenic assays has also been shown to be limited (27, 40). Metabolic activation activity for the enzyme in microsomes from human gastrointestinal tract has been reported but only at low levels (38).

In more extensive experiments with purified P450 1A2 and varying substrate concentrations, kinetic parameters (K_m and V_max) for the N-hydroxylation of several arylamines were determined (Table II). MeIQx was not included because the specific activity of our radiolabelled sample was too low to permit measurement of the N-hydroxy product at low substrate concentrations. The K_m values for ABP (30 µM), IQ (33 µM), and PhIP (46 µM) were similar. Values for V_max were also similar (1.8–4.2 nmol/min/nmol P450). The K_m value for the N-hydroxylation of PhIP is consistent with the value (55 µM) previously observed with human liver microsomes (26).

Since furafylline (41) and fluvoxamine (42) have been shown to be inhibitory to P450 1A2 activity in humans, the K_i values for these inhibitors with the purified recombinant P450 1A2 enzyme were, therefore, assessed. N-Hydroxylation

### Table I. Comparative rates of N-hydroxylation of arylamines by recombinant human cytochromes P450a

<table>
<thead>
<tr>
<th>P450</th>
<th>nmol/min/nmol P450</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABP</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressed in E.colib</td>
<td></td>
</tr>
<tr>
<td>P450 1A1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>P450 1A2</td>
<td>3.80 ± 0.45</td>
</tr>
<tr>
<td>P450 3A4</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Expressed in human B-lymphoblastoid cellsb</td>
<td></td>
</tr>
<tr>
<td>P450 1A1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>P450 1A2</td>
<td>1.98 ± 0.13</td>
</tr>
</tbody>
</table>

aThe values are the means of 2 or 3–5 determinations ± SD.
bEnzymes expressed in E.coli were purified and assayed in reconstituted systems; those expressed in human B-lymphoblastoid cells were assayed as microsomal preparations.
cRate of 4'-hydroxylation.
dnd, not determined.

### Table II. Kinetic parameters of N-hydroxylation of arylamines by purified recombinant human P450 1A2 expressed in E. coli

<table>
<thead>
<tr>
<th></th>
<th>K_m (µM)</th>
<th>V_max (nmol/min/nmol P450)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABP</td>
<td>30 ± 5</td>
<td>4.19 ± 0.19</td>
</tr>
<tr>
<td>IQ</td>
<td>33 ± 2</td>
<td>1.81 ± 0.02</td>
</tr>
<tr>
<td>PhIP</td>
<td>46 ± 4</td>
<td>1.79 ± 0.05</td>
</tr>
</tbody>
</table>

The values are means ± SEM of the data set using a non-linear kinetic analysis from mean values obtained in duplicate at each substrate concentration.
activity was measured under conditions in which the substrate and inhibitor were co-incubated. Dixon plots were constructed to determine the reversible binding constant $K_i$ (Figure 1). Respective $K_i$s, estimated from the reciprocal plots of velocity versus inhibitor concentration, were $\sim 15 \mu M$ and $\sim 5 \mu M$ for furafylline and fluvoxamine. A $K_i$ value of $\sim 3 \mu M$ has been reported for furafylline inhibition of phenacetin O-deethylase activity of human liver microsomes (43). In other studies with furafylline, under conditions of preincubation in the presence of NADPH, it has been shown to be a mechanism-based inhibitor of P450 1A2 activity in lysates of COS-7 cells expressing human P450 1A2 (44) and in human liver microsomes (43,45,46). Values of $K_i$ determined under these conditions, for expressed P450 1A2 (6.9 $\mu M$) and human liver microsomes (0.6–23 $\mu M$) were also similar. Fluvoxamine was found to competitively inhibit phenacetin O-deethylase in a V79 cell line expressing human P450 1A2 with a $K_i$ of 2.7–14.5 nM (47). These values are much lower than that determined in our study. The reasons for the apparent difference are uncertain.

In conclusion, our results demonstrate that human P450 1A2 effectively catalyzes the N-hydroxylation of ABP and food-borne heterocyclic amine carcinogens. Enzyme expressed in either of two different systems was similarly active with some variation. Little or no activity with the arylamines included in this study was observed with P450 1A1 or P450 3A4. These results are consistent with previous studies in which human P4501A2 was a much more effective catalyst in activating heterocyclic amines to mutagenic products compared with other P450 enzymes (27,40). Support is thereby provided for the P4501A2-catalyzed N-hydroxylation activation pathway in humans implicated by other studies (22,23,26,48) and further suggesting that individual levels of P450 1A2 play a major role in determining risk to the carcinogenicity of these arylamines.

References

Fig. 1. Dixon-plot analyses of the effect of furafylline (A) and fluvoxamine (B) on N-hydroxylation of ABP by purified recombinant human P450 1A2. ABP concentrations: (□) 0.01 mM; (●) 0.025 mM; (○) 0.05 mM; (●) 0.1 mM.


Received on October 31, 1996; revised on November 26, 1996; accepted on December 6, 1996.