Oxidation of Benzo[a]pyrene by Recombinant Human Cytochrome P450 Enzymes

Eckhart Bauer,[†,‡] Zuyu Guo,[†] Yune-Fang Ueng,[†] L. Chastine Bell,[†] Darryl Zeldin,[§] and F. Peter Guengerich*

Department of Biochemistry and Center in Molecular Toxicology, and Division of Nephrology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

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The oxidation of benzo[a]pyrene (B[a]P) was examined using reconstituted systems prepared with recombinant human cytochrome P450 (P450) enzymes 1A1, 1A2, 2C8, 2C10, 2E1, and 3A4 and with microsomes prepared from *Saccharomyces cerevisiae* expressing recombinant human P450s 2C8, 2C9, and 2C18. Products measured by HPLC included the 3- and 9-phenols, the 4,5-, 7,8-, and 9,10-dihydrodiols (detected in the presence of epoxide hydrolase), and products in the polar fraction eluting immediately after the void volume. The most active enzyme in all reactions was P450 1A1. P450 3A4 and P450 1A2 formed appreciable amounts of several of the products, including the 3-phenol. P450 2C enzymes and P450 2E1 formed relatively low amounts of all B[a]P products. Consideration of these patterns along with knowledge of levels of expression of the P450s in human tissues and previous results with microsomes leads to the conclusion that P450 1A1 should dominate the oxidation of B[a]P in tissues where it is present and inducible. In human liver the level of P450 1A1 is low and P450 3A4, P450 2C subfamily enzymes, and P450 1A2 probably all contribute. Of the human P450s considered here, P450 1A2 was the most active hepatic enzyme forming the 7,8-dihydrodiol. 7,8-Benzoflavone stimulated the oxidation of B[a]P by P450 3A4 and inhibited the oxidations catalyzed by P450 1A2. The extent of inhibition of P450 1A1 was less (than with P450 1A2), probably due to the rapid oxidation of 7,8-benzoflavone by P450 1A1. The major 7,8-benzoflavone product appears to be the 5,6-oxide.

Introduction

Large amounts of benzo[a]pyrene (B[a]P)[1] are released into the atmosphere from combustion processes; the estimated annual release in the United States is ~8×10^5 kg (*5*). Humans also ingest this and other related polycyclic hydrocarbons from cigarette smoke and food (*5, 6*). The compound has long been known to be tumorigenic in experimental animals and has served as a prototype for chemical carcinogens (*7, 8*). Biotransformation of B[a]P has been known to be necessary for its conversion to a form reactive enough to bind to DNA and initiate tumors, and the elucidation of the key events in the conversion of B[a]P to diol epoxides is now classic in the field of chemical carcinogenesis (*6, 9-13*).

Many studies have been done on the rat liver cytochrome P450 (P450) enzymes involved in the oxidation of B[a]P (*13-16*). Less attention has been given to the human P450 enzymes, particularly those expressed in liver. For a number of years it has been known that B[a]P 3-hydroxylation and the oxidation of B[a]P-7,8-dihydrodiol to a mutagen can be directly stimulated by the addition of 7,8-benzoflavone (α-naphthoflavone, αNF) in human liver microsomes (*17-19*). A number of lines of evidence indicate that the level of P450 1A1, which has received the bulk of attention in rodent studies (*10*), is expressed at only very low levels in the majority of (adult) human livers (*20-22*). McManus *et al.* (*23*) showed that human P450 1A1 and P450 1A2, expressed in COS kidney cells, were able to oxidize B[a]P to more polar (radioactive) products, with P450 1A1 being considerably more active than P450 1A2. Studies in this laboratory have indicated a significant role for P450 3A4 in the 3-hydroxylation of B[a]P (*24*) and the 9,10-epoxidation of B[a]P-7,8-dihydrodiol (*25*). We also reported some evidence for a partial contribution of P450 2C enzymes in hepatic B[a]P 3-hydroxylation (*24*) and reviewed earlier literature in the area. A major deficiency, however, has been the inability to identify specific enzymes involved in the low levels of B[a]P 3-hydroxylation and 7,8-epoxidation seen in many human liver samples (*24, 25*).

With the exception of the study of McManus *et al.* (*23*) with human P450s 1A1 and 1A2 expressed in COS cells, little has been published on the activities of recombinant human P450 enzymes toward B[a]P, at least in terms of specific products. In that work only total extractable polar products were measured. Roberts-Thomson *et al.* (*26*) examined human P450s 1A1, 1A2, 3A4, and 3A5 (expressed in COS cells), but the results are somewhat equivocal since no quantitation of P450 expression was done. The rates of product formation were actually rather similar, on a cell protein basis, for all four

* Author to whom correspondence and reprint requests should be addressed at the Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232-0146.

† Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146.

‡ Present address: Department of Drug Metabolism and Pharmacokinetics, Dr. Karl Thomae GmbH, 88397 Biberach, Federal Republic of Germany.

§ Division of Nephrology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232. Present address: National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC.

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[1] Abbreviations: P450, cytochrome P450; B[a]P, benzo[a]pyrene; b_5, cytochrome b_5; αNF (α-naphthoflavone), 7,8-benzoflavone. For reference to the nomenclature of P450 enzymes see ref 1, and for recent reviews of human P450 enzymes see refs 2-4.

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enzymes. This result seems surprising in light of the expectation of high activity for P450 1A1. Human P450 1A1 has been expressed in a variety of other heterologous vector systems, including yeast. In most of these either no measurements of B[*a*]P oxidation were made (*27*) or else only 3-hydroxylation was measured (*28*). Gautier *et al.* (*29*) also expressed human P450 1A1 in *Saccharomyces cerevisiae* and detected the 3- and 9-phenols and 7,8-dihydrodiol from B[*a*]P, in the presence of expressed epoxide hydrolase. Comparisons were not made with other human P450s in that work. Doehmer *et al.* (*30*) measured products derived from Chinese hamster ovary cells expressing human P450 1A1 (over a 24 h period) and apparently found little 3-hydroxy-B[*a*]P, with the bulk of the detected products resulting from oxidation at carbons 7, 8, 9, and 10. Similar results were seen with rat P450 1A1 in such a system and are not consonant with the known literature of these enzymes using systems where initial rates of oxidation are measured (*13, 31–34*).

Recently we have been able to express human P450s 1A1, 1A2, 2C10, 2E1, and 3A4 in *Escherichia coli* with slight modification of the 5′-termini and purify the proteins (*35–39*). We have used reconstituted systems containing these P450s to examine their roles in B[*a*]P oxidation, along with purified P450 2C8 produced in baculovirus and yeast microsomes containing recombinant P450s 2C8, 2C9, and 2C18.

Experimental Procedures

Caution: *The following chemicals are hazardous and should be handled carefully: benzo[a]pyrene itself and all benzo[a]-pyrene derivatives. Protective clothing should be worn. Crystalline material presents an inhalation hazard because the crystals develop electrostatic charge and cling to dust particles.*

Chemicals. All operations with B[*a*]P were done in amber glass vessels or under subdued light. [1,3,6-^{3}H]B[*a*]P (63 Ci mmol^{-1}) was purchased from DuPont/New England Nuclear Corp. (Boston, MA) and purified by passing a hexane solution through a short (5 × 20 mm) column of silica gel G, eluting with hexane. The hexane was removed *in vacuo*. HPLC analysis with the analytical system used for analysis of incubations indicated a radiochemical purity of >99.3%. The stock material was routinely diluted to 0.50 μCi μmol^{-1} for use in incubations. Standard B[*a*]P products were obtained from the National Cancer Institute Chemical Repository through Midwest Research Institute (Kansas City, MO) and were used without further purification. 7,8-Benzoflavone (α-naphthoflavone, αNF) was purchased from Aldrich Chemical Co. (Milwaukee, WI). GSH was purchased from Sigma Chemical Co. (St. Louis, MO).

Enzymes. NADPH–P450 reductase was purified from livers of phenobarbital-treated rabbits as described by Yasukochi and Masters (*40*), with slight modification. Hydroxylapatite chromatography was used to remove detergent at the last step, instead of gel filtration. Cytochrome b_5 (b_5) was purified from human liver microsomes (*41, 42*). Epoxide hydrolase was purified from liver microsomes of phenobarbital-treated rats (*43*).

Human P450s 1A1 (*39*), 1A2 (*37*), 2C10 (*36*), 2E1 (*38*), and 3A4 (*35*) were expressed in *E. coli* using the pCW vector and purified to electrophoretic homogeneity as described. The modifications that were made to the 5′ terminal sequence are described in the references. P450 2C8 was expressed in a baculovirus system and purified using a procedure similar to that used for P450 2C10 (*36*), which will be presented in detail elsewhere.[2] P450s 2C8, 2C9, and 2C18 were expressed in *S. cerevisiae* (*44–46*)[3] and were kindly provided by Drs. J.-C. Gautier, J. Cosme, D. Pompon, and P. H. Beaune (Necker-INSERM 75, Paris, France). Microsomes were prepared from these yeasts and used directly in B[*a*]P oxidation assays. All of the yeast microsomes expressing P450 2C enzymes were active in tolbutamide methyl hydroxylation (*36, 44, 46*).[3]

B[*a*]P Oxidation Assays. Optimal conditions vary among the individual human P450s and were developed using other reactions with the same enzyme preparations: 1A1 and 1A2, 7-ethoxyresorufin O-deethylation (*39*); 2C10, tolbutamide methyl hydroxylation (*36*); 2E1, chlorzoxazone 6-hydroxylation (*38*); 3A4, nifedipine oxidation, testosterone 6β-hydroxylation, and aflatoxin B_1 3α-hydroxylation and 8,9-epoxidation (*35, 47*).

In all cases, including work with the yeast microsomes, the B[*a*]P concentration was 40 μM and epoxide hydrolase was added at 0.5 μM. Incubations were generally done at 37 °C for 30 min, except for P450s 1A1 and 1A2 (10 min). With all P450s except P450 2E1 and 3A4, the buffer mixture included 25 mM Tris-HCl (pH 7.4), 150 mM KCl, and 10 mM $MgCl_2$. With P450 2E1, the buffer was 100 mM potassium phosphate (pH 7.4). The P450 3A4 buffer system is described later.

With purified P450s 1A1, 1A2, 2C8, and 2C10, 100 pmol of P450 was mixed with 200 pmol of NADPH–P450 reductase and 15 nmol of L-α-1,2-dilauroyl-*sn*-glycero-3-phosphocholine (lipid dispersed by sonication in H_2O at 1 mg mL^{-1}), and the components were allowed to stand 10 min at 23 °C before H_2O, the buffer mixture, epoxide hydrolase, and B[*a*]P were added. The mixture was preincubated for 3 min at 37 °C and then initiated by the addition of an NADPH-generating system (*46*). In all cases the final reaction volume was 250 μL. In the case of some of the experiments with P450 2C10, b_5 was added (100 pmol) in the first preincubation mixture. With P450 2E1, the system included 200 pmol of b_5 and 300 pmol of NADPH–P450 reductase, and the final volume was 500 μL.

With P450 3A4, 50 pmol of the purified enzyme was mixed with 50 pmol of b_5, 100 pmol of NADPH–P450 reductase, 10 μg of a 1:1:1 (w/w) dispersed aqueous mixture of L-α-dilauroyl-*sn*-glycero-3-phosphocholine, L-α-dioleyl-*sn*-glycero-3-phosphocholine, and bovine brain phosphatidylserine (all from Sigma), and 1.5 μmol of GSH (dissolved at 0.15 M in 0.10 M potassium phosphate buffer, pH 7.4). This mixture (~90 μL) was then incubated for ~10 min at room temperature. The following components were then added in order to give the final indicated concentrations (total volume 250 μL): 50 mM potassium *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonate (HEPES) (pH 7.85), 30 mM $MgCl_2$, (H_2O), 40 μM B[*a*]P, and (following an additional 3 min preincubation at 37 °C) an NADPH-generating system (*47, 48*).

The incubations with the reconstituted enzymes were terminated by rapid freezing of the samples in dry ice/$(CH_3)_2CO$. B[*a*]P, its metabolites, and the added standards were extracted by direct injection of the sample onto a small extraction column (4.6 × 20 mm, dry filled with Corasil C18, 37–50 μm, Waters, Milford, MA). This extraction column was connected to the 6-port injection valve (Rheodyne 7125) of the HPLC instead of a normal injection loop. The extraction column was washed with 3 mL of water (with the eluate diverted to waste), and the extract was transferred by the gradient eluent onto the analytical column in the opposite direction. The octadecylsilane analytical HPLC column (4.6 × 125 mm plus 4.6 × 20 mm guard column, Thomae, Biberach, Germany) had been equilibrated with a CH_3OH–5 mM potassium phosphate (pH 6.8) mixture (55:45 v/v). The CH_3OH concentration was increased to 100% (*16, 24, 25*) over 50 min with a linear gradient (flow rate 1.0 mL min^{-1}). The HPLC pumping system was a Model P200 (Thermo Separation Products, Piscataway, NJ). The effluent passed sequentially through three detectors: (i) UV (Model Spectraseries UV 100, Thermo Separation Products), A_{254}; (ii) fluorescence (Model FL-750 BX, McPherson Instrument, Acton, MA), $F_{344/418}$; (iii) radioactivity (β-RAM, IN/US, Tampa, FL), with

[2] D. Zeldin and J. H. Capdevila, in preparation.

[3] J.-C. Gautier, J. Cosme, D. Pompon, and P. H. Beaune, in preparation.

mixing with Flo-Scint III cocktail (Packard Instruments, Downers Grove, IL) pumped at 3.0 mL min^{-1}. The output from all three detectors was directed to a DOS-based computer system (IN/US) and processed using that system.

The reactions with microsomes were terminated by the addition of 0.25 mL of $(CH_3)_2CO$, and the products were extracted twice with 0.50 mL of ethyl acetate by mixing with the use of a vortex device. The organic layers were transferred to clean tubes and reduced to dryness under a N_2 stream. Each sample was dissolved in 20 μL of CH_3OH containing a mixture of product standards, and 10 μL was injected directly onto the guard/analytical column combination. Elution was as described for the reconstituted enzyme systems.

Results

Oxidation of B[*a*]P by Individual P450s. Representative HPLC traces for B[*a*]P oxidation are presented in Figure 1. All major radioactive peaks were verified by their characteristic retention times and by comparisons with the UV and fluorescence traces resulting from the added standards. The traces seen in the absence of NADPH were essentially completely devoid of any of the products under consideration (Figure 1E). Quinones are often generated from B[*a*]P oxidation products (*16, 31, 49*); the levels of these were rather low in all cases.

The results are summarized in Table 1. P450 1A1 was the most active in generating all of the B[*a*]P products. However, the rates of total B[*a*]P oxidation appear to be less than those reported for the orthologous rabbit (*15*) and rat (*16*) P450 1A1 enzymes.

Two other P450 enzymes had relatively high rates of oxidation of B[*a*]P to several products, P450 1A2 and P450 3A4 (Table 1). P450 2E1 and the purified recombinant bacterial P450 2C10 had low but (some) measurable activities (Figure 1); purified recombinant baculovirus P450 2C8 had no activity toward B[*a*]P. In order to further assess the activity of P450 2C enzymes, we also utilized microsomal samples prepared from yeast in which recombinant P450s 2C8, 2C9, and 2C18 were expressed (Table 1). These preparations (also checked for tolbutamide hydroxylation) showed low rates of oxidation of B[*a*]P.

P450s 2E1 and 3A4 are highly dependent upon the presence of b_5 in these systems using other substrates (*35, 38*), and we did not attempt to measure B[*a*]P oxidation without b_5. Some b_5 stimulation of P450 2C10 was seen (b_5 has been found to inhibit P450 2C8).[2] The removal of GSH from the P450 3A4 system did not produce a noticeable effect on 3-hydroxylation, but it did decrease rates of formation of the 4,5- and 9,10-dihydrodiols and increase the level of 7,8-dihydrodiol, and the polar fraction eluted immediately after the void volume of the column.

Effects of αNF on B[*a*]P Oxidation. αNF (5 μM initial concentration) inhibited the formation of some of the products of B[*a*]P formed by P450s 1A1 and particularly P450 1A2, as expected from the literature (*23, 50*). However, the extent of inhibition of individual reactions catalyzed by P450 1A2 was not uniform (e.g., 4,5-dihydrodiol formation). The same pattern was seen with P450 1A1, and 9,10-dihydrodiol formation was actually increased. One of the reasons that αNF did not inhibit P450 1A1 more extensively was the rapid metabolism of this compound (by P450 1A1). The reaction time had to be restricted to <10 min under these conditions to observe inhibition. The major αNF product in the absence of epoxide hydrolase (Figure 2) was characterized as the 5,6-oxide on the basis of its UV (λ_{max} 270, 300 nm in CH_3OH) and mass spectral properties (fast atom bombardment, glycerol/Me_2SO/CF_3CO_2H mixture, m/z 289 $[M + H]^+$, relative abundance 62) and comparison to the literature (*51, 52*).

In the presence of epoxide hydrolase the major product detected was more polar, apparently the 5,6-dihydrodiol. The 5,6-oxide has been identified as the major αNF oxidation product of P450 3A4 (in the absence of epoxide hydrolase) (*47*). The same product was formed by P450 1A2 but at a rate ~1/10 that of P450 1A1.

In other experiments, αNF (30 μM) was found to stimulate the oxidation of B[*a*]P by P450 3A4, as expected from previous work (*17, 18, 23*–25, 53). Rates of oxidation of B[*a*]P to the 4,5- and 7,8-dihydrodiols and 3- and 9-phenols were increased ~2-fold (in all cases), and the amount of residual B[*a*]P was also decreased ~2-fold under these conditions.

Discussion

Our results indicate that P450 1A1 is the most active of all human P450s examined in forming all of the B[*a*]P oxidation products with the exception of the 4,5-dihydrodiol and the uncharacterized polar material eluting with the front, where P450 1A2 had similar activity (Table 1). The results were highly reproducible among different bacterial P450 preparations examined. The reconstitution conditions were developed for other reactions catalyzed by these enzymes and appear to be reasonably optimal. Also, the sensitivity of the HPLC system was excellent because of the low background (Figure 1E). Products migrating in the quinone region of the HPLC chromatograms are often associated with artifacts (arising from nonenzymatic oxidation before or after incubation) and were nearly absent in our work (Figure 1, Table 1).

These results may be compared to others in the literature. In agreement with McManus *et al.* (*23*) we find relatively high B[*a*]P 7,8-oxidation for P450 1A1 and some for P450 1A2. We also agree with Roberts-Thomson *et al.* (*26*) that P450s 3A4 and 1A2 have the abilities to oxidize B[*a*]P, but our rates for oxidations do not follow the same order they reported. It is highly probable that those results are the reflection of varying levels of expression of different P450s in the cells, given the high B[*a*]P oxidation activity of human P450 1A1 seen here (Table 1).

Comparisons can also be made with work done in this laboratory with human liver microsomes and P450s isolated from them (*24, 25*). The results are in general agreement regarding the role of P450 3A4 in B[*a*]P 3-hydroxylation (*24*), since the recombinant enzyme showed considerable activity (Table 1), higher than the rates measured in most human liver microsomes (*24*). We previously reported that P450 2C enzymes had some role in B[*a*]P 3-hydroxylation in human liver, on the basis of studies involving inhibition by sulfaphenazole and anti-P450 2C antibodies and reconstitution of a P450 2C8 protein purified from human liver (*24*). Others had concluded that P450 2C enzymes have a major role in human liver B[*a*]P 3-hydroxylation on the basis of correlation and other studies (*54, 55*), although we have been of the opinion that P450 3A4 has a generally more major role in this reaction (*24*). In the present work we find that all of the recombinant human P450 2C enzymes examined seem to have low B[*a*]P 3-hydroxylation activi-

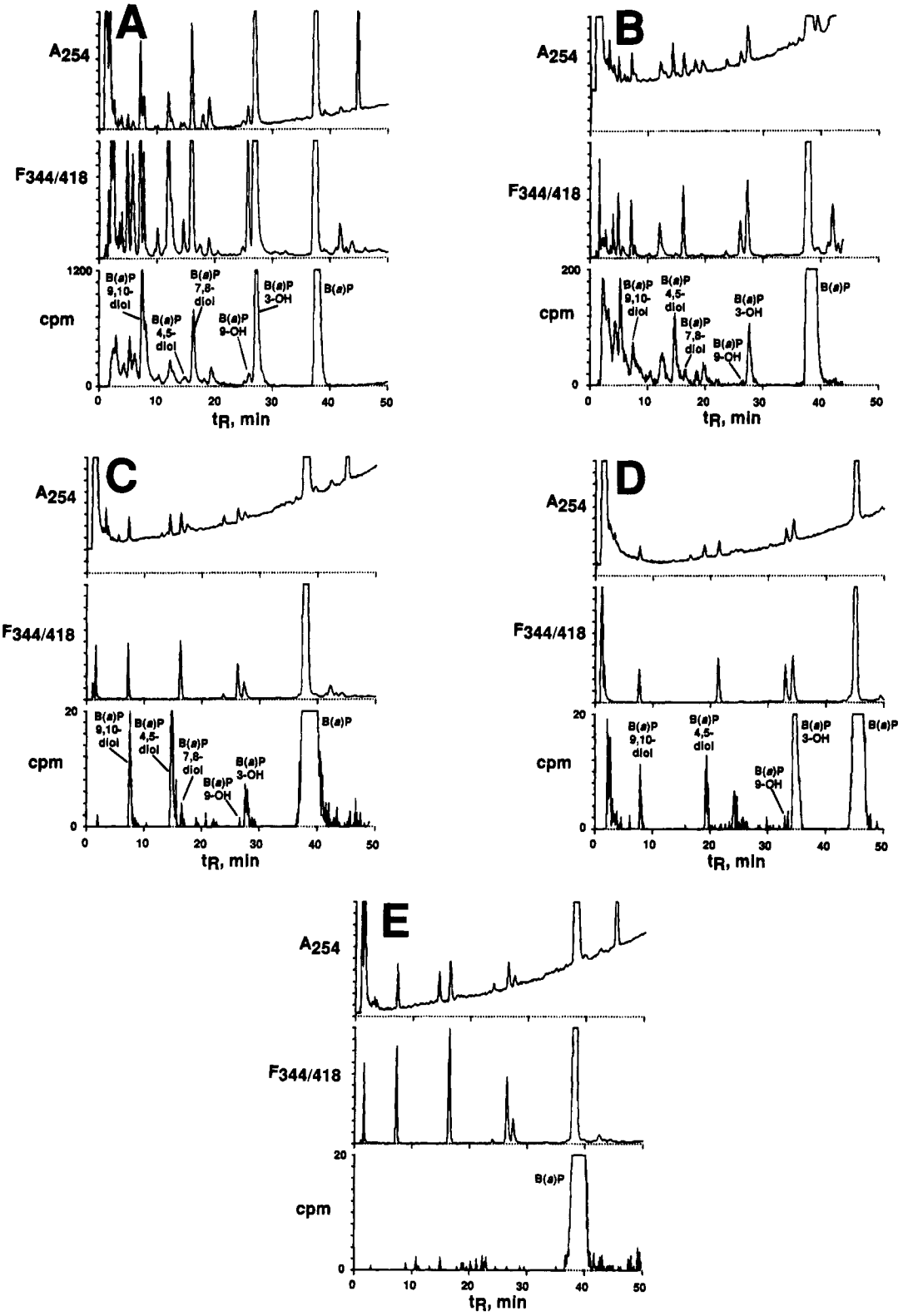

Figure 1. HPLC of B[*a*]P oxidation products by recombinant human P450s. (A) P450 1A1, (B) P450 1A2, (C) P450 2C10, (D) P450 3A4. In each part are shown the (a) UV trace (upper), (b) fluorescence trace (middle), and (c) radioactivity trace (^{3}H, lower). In part E a trace from an incubation is shown where the P450 2C10 was incubated in the absence of NADPH. The standard products added to the analytes included 3-hydroxy-B[*a*]P (B[*a*]P-3-OH), 9-hydroxy-B[*a*]P (B[*a*]P-9-OH), *trans*-4,5-dihydroxy-4,5-dihydro-B[*a*]P (B[*a*]P-4,5-diol), *trans*-7,8-dihydroxy-7,8-dihydro-B[*a*]P (B[*a*]P-7,8-diol), and *trans*-9,10-dihydroxy-9,10-dihydro-B[*a*]P (B[*a*]P-9,10-diol).

ties, regardless of the expression system used (Table 1). The previously reported B[*a*]P 3-hydroxylation activity of P450 2C8 (*24*) does not seem to be supported by the current work with the recombinant systems (Table 1). Assignment of the previously used hepatic P450 2C8 was based upon partial amino acid sequence analysis (*24*). It is conceivable that the discrepancy is due to small differences in protein structure (e.g., the existence of very closely related genes in the P450 2C families or allelic variants) or the contamination of the hepatic P450 2C8 preparation with traces of other P450s.

After submission of our manuscript a report by Shou *et al.* (*56*) compared the B[*a*]P oxidation activities of recombinant human P450s 1A1 and 1A2 expressed in

Table 1. Oxidation of B[a]P by Recombinant Human P450s

P450	b_5	GSH	αNF	product formed, pmol min^{-1} (nmol of P450)$^{-1}$ [a] front	9,10-diol	4,5-diol	7,8-diol	quinones	9-OH	3-OH	% residual B[a]P
1A1	−	−	−	1210	496	108	594	108	60	708	75
1A1	−	−	+	670	1080	100	506	92	20	450	82
1A2	−	−	−	1002	23	104	26	44	20	164	90
1A2	−	−	+	260	<1	54	<1	<1	<1	1	97
2C8	−	−	−	<1	<1	<1	<1	<1	<1	<1	>99
2C10	−	−	−	<1	3	6	5	1	<1	3	>99
2C10	+	−	−	<1	9	18	1	1	<1	5	99
2E1	+	−	−	27	<1	<1	2	<1	1	17	98
3A4	+	−	−	7	12	23	3	17	7	243	95
3A4	+	+	−	70	20	35	<1	29	5	235	94
2C8[b]		−	−	17	<1	15	5	<1	<1	5	98
2C9[b]		−	−	1	<1	<1	2	<1	<1	11	99
2C18[b]		−	−	<1	<1	<1	1	<1	<1	22	99

[a] Results are presented as means determined with duplicate incubations. The results differed by <15%. [b] Yeast microsomes (*29, 45, 46*).[3]

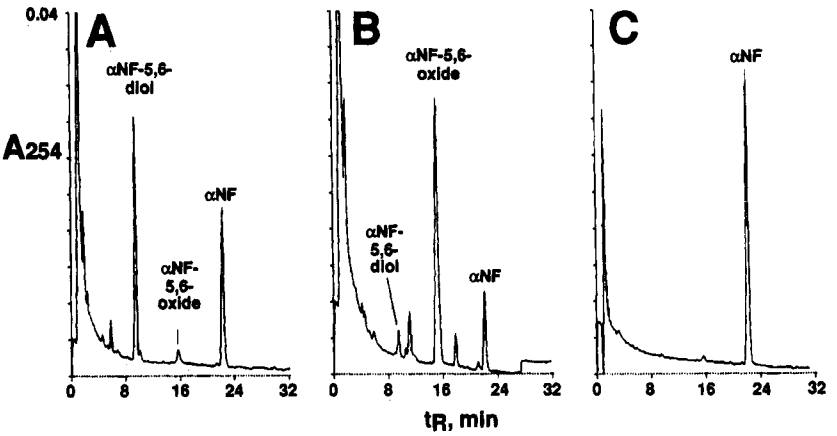

Figure 2. Products of incubation of αNF (5 μM) with recombinant human P450 1A1. P450 1A1 (50 pmol) in the usual reconstituted enzyme system (250 μL) was incubated in (A) the presence and (B) the absence of rat liver epoxide hydrolase (0.5 μM). In part C no epoxide hydrolase or NADPH-generating system was added. A linear gradient of 50−100% CH_3OH (in H_2O, over 30 min) was used to elute the HPLC column (flow 1.0 mL min^{-1}). αNF-5,6-diol indicates αNF-5,6-dihydrodiol.

human lymphoblastoid cells and human P450s 1A2, 2B6, 2C8, 2E1, and 3A4 expressed with vaccinia virus in HepG2 cells. Many of the results are qualitatively similar to those we report here with purified and reconstituted bacterial recombinant human P450s. As in our study, P450 1A1 was clearly the most active enzyme. The rates of certain B[a]P oxidations found by Shou *et al.* (*56*) for P450s 2C9 and 2E1 are somewhat higher than our values (Table 1). On the other hand, their absolute rates for most of the oxidations catalyzed by P450s 1A1, 1A2, and particularly 3A4 are considerably lower than ours so that the ordering of activities of the P450s is different, particularly regarding the roles of P450s 1A2 and 3A4. The direct comparison of rates in different expression systems is not trivial due to requiements for b_5, NADPH−P450 reductase, etc., and the use of tissue studies is needed to properly ascertain roles of various P450s (*vide supra*).

Another aspect of B[a]P oxidation we considered previously was the roles of P450 enzymes involved in the 7,8-epoxidation of B[a]P (*25*). In human liver microsomes this reaction seems to be inhibited by αNF, but a hepatic P450 1A2 preparation did not catalyze the reaction at detectable rates, and we then attributed the reaction to traces of P450 1A1 in human liver (*25*). However, we now find appreciable activity with recombinant P450 1A2, considerably more than with P450 3A4 or any of the P450 2C or other (i.e., P450 2E1) enzymes known to be expressed in human liver. Thus, P450 1A2 probably seems to be a reasonable assignment to B[a]P 7,8-epoxidation in human liver, a critical step in the formation of B[a]P-7,8-dihydrodiol-9,10-epoxide.

Allelic variants of P450 1A1 have been reported and considered with regard to lung cancer risk (*57, 58*). The particular P450 1A1 cDNA used here, originally obtained from Dr. R. H. Tukey (*59*), has Ile at residue 462. Another prominent allele has a Val at this position (*57*). The polymorphism does not appear to be linked to an *Msp*I polymorphism, at least in Caucasians (*58, 60*). A 2-fold difference in catalytic activity toward B[a]P has been reported (*61*), but the significance with regard to lung cancer incidence is unknown.

It should also be emphasized that studies with αNF must be interpreted carefully. In our studies we found that αNF was rapidly oxidized by P450 1A1 (and, to a lesser extent, by P450 1A2) and was less effective as an inhibitor in prolonged incubations. The effects of this inhibitor may easily be overlooked in some systems, particularly when the rate of oxidation of αNF is greater than that of the substrate under consideration.

In summary, P450 1A1 is the most active of any of the human P450s considered here for all of the oxidation reactions. With regard to B[a]P 3-hydroxylation, P450 3A4 seems to play a prominent role, as judged by the rates measured with the purified enzyme, the high abundance of P450 3A4 (*62, 63*), and previous correlation and inhibition studies (*24, 25*). P450 2C subfamily enzymes seem to contribute somewhat. The intrinsic rates are low (Table 1), but the abundance of the entire class of P450 2C subfamily enzymes as a whole (*62*) and

previous inhibition studies (*24*) indicate that they do have a contributing role in B[*a*]P oxidation, at least in some livers. P450 1A2 also has a role in several of the oxidation reactions, particularly to B[*a*]P 7,8-epoxidation (Table 1). At this time we should conclude, on the basis of earlier inhibition studies (*25*) and the work presented here, that P450 1A2 is a major contributor to B[*a*]P 7,8-epoxidation in human liver.

In general, B[*a*]P 7,8-epoxidation is considered an activation reaction, and most of the other reactions are considered detoxications (*6, 9–11*). Thus, P450 1A2 might be considered a "bioactivating" enzyme. However, P450 1A1 catalyzes both activation (e.g., 7,8-epoxide formation) and detoxication pathways (e.g., 3-hydroxylation). P450 3A4 primarily catalyzes apparent detoxication of B[*a*]P although it can have a signifcant role in the activation of the 7,8-dihydrodiol (*25*). With the P450 2C enzymes, the rates are low and defy categorization. However, it is possible that allegedly detoxicated B[*a*]P products are parts of unknown overall activation schemes that have not been fully recognized yet.

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