# Stereoselective Epoxidation and Hydration at the K-Region of Polycyclic Aromatic Hydrocarbons by cDNA-expressed Cytochromes P450 1A1, 1A2, and Epoxide Hydrolase

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ABSTRACT: Stereoselective epoxidation by cytochrome P450s (P450s) and regioselective hydration by epoxide hydrolase determine the carcinogenic potency of some polycyclic aromatic hydrocarbons (PAHs). In this report, cDNA-expressed human and mouse P450s 1A1 and 1A2 and epoxide hydrolase were used to characterize the stereoselective epoxidation and regioselective hydration at the K-region of benz[*a*]-anthracene (BA), 7,12-dimethylbenz[*a*]anthracene (DMBA), chrysene (CR), benzo[*a*]pyrene (B[*a*]P), dibenz[*a,h*]anthrancene (DB[*a,h*]A), and benzo[*c*]phenanthrene (B[*c*]Ph) by direct chiral stationary-phase HPLC (CSP-HPLC) analyses. Our results indicated that all P450 isoforms preferentially produced major K-region *S,R*-epoxides of BA (95−98%), DMBA (94−97%), B[*a*]P (91−96%), DB[*a,h*]A (94−98%), and B[*c*]Ph (87−92%), and major *R,S*-epoxide of CR (74−85%) in the presence of 3,3,3-trichloropropylene 1,2-oxide (TCPO), an inhibitor of epoxide hydrolase, suggesting that P450 enzymes exhibited the high stereoselectivity toward one of two stereoheterotopic faces of K-region double bond of the PAHs. Epoxide hydrolase either expressed from recombinant vaccinia virus or contained in human hepatoma G2 cells (HepG2) hydrated the C−O bond of epoxy-ring at the *S*-carbon of major metabolically-formed K-region epoxide enantiomers of BA, CR, DMBA, B[*a*]P, and DB[*a,h*]A to yield 80−98% dihydrodiols enriched in *R,R*-form and that at the *R*-carbon of B[*c*]Ph epoxide to yield 77−92% dihydrodiol enriched in *S,S*-form, suggesting that epoxide hydrolase was highly regioselective. The various enantiomeric components of dihydrodiol products in the metabolism of PAHs were apparently due to the combined effect of stereoselectivity of the P450s and regioselectivity of epoxide hydrolase. Our results provide a clear understanding of how these enzymes catalyze overall stereoselective metabolism at the K-region of the PAHs.

Polycyclic aromatic hydrocarbons (PAHs)[1] are common particulate environmental pollutants, and some of them have been reported to be carcinogenic in experimental animals and humans (Gelboin, 1980; Conney, 1982; Dipple, 1985). The biological properties of PAHs, such as mutagenicity, carcinogenicity, and covalent binding to cellular macromolecules, require stereoselective metabolic activation by cytochromes P450 and epoxide hydrolase (Jerina et al., 1978, 1982; Conney, 1982; Yang et al., 1985a, 1988; Oesch, 1972; Lu et al., 1977). Cytochrome P450 is a superfamily of enzymes that metabolize a variety of endogenous and exogenous compounds (Gonzalez et al., 1989, 1991; Guengerich & Shimada, 1991). P4501A subfamily enzymes in different species such as human 1A1 are responsible for metabolic activation of PAHs (Gelboin, 1980; Conney, 1982; Shou et al., 1994a; Bauer et al., 1995). Epoxides are the initial products of PAHs by P450 enzymes which are further converted to *trans*-dihydrodiols by microsomal epoxide hydrolase (Jerina et al., 1972; Yang, 1985a, 1988). If *trans*-dihydrodiol occurs at the angular benzo-ring of a PAH (M-region), it may undergo a vicinal epoxidation of the *trans*-dihydrodiol by P450s to the bay-region diol epoxides which are believed as the ultimate carcinogens.

Due to stereoheterotopic interactions between the substrate and cytochrome P450 enzyme (selective toward one of the two stereochemically different faces), some metabolically-formed epoxides are optically active (Yang, 1988). Epoxide hydrolase that opens the C−O bond of the epoxy-ring is regioselective toward one of two chiral carbons of an optically pure epoxide enantiomer and enantioselective toward one of a pair of epoxide enantiomers, resulting in *trans*-dihydrodiol with various enantiomeric composition. For example, benzo[*a*]pyrene (B[*a*]P) is metabolically activated predominantly to the 7*R*,8*S*-dihydrodiol-9*S*,10*R*-epoxide (*anti* form) via 7*R*,8*S*-epoxide and 7*R*,8*R*-dihydrodiol. This diol-epoxide is significantly more mutagenic and carcinogenic than the other optically active diol-epoxides that are formed metabolically (Buening et al., 1978; Levin et al., 1980). Thus, both P450s and epoxide hydrolase are involved in the stereoselective activation of a parent PAH to the ultimate carcinogen diol-epoxides.

When epoxide hydrolase is inhibited by using an inhibitor such as 3,3,3-trichloropropylene 1,2-oxide (TCPO), epoxides formed in the metabolism of PAHs, if sufficiently stable, can be successfully isolated by normal-phase HPLC (Yang et al., 1985b, 1987a) and the enantiomeric ratio of epoxides

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[1] Abbreviations: PAH, polycyclic aromatic hydrocarbon; B[*a*]P, benzo[*a*]pyrene; BA, benz[*a*]anthrancene; CR, chrysene; B[*c*]Ph, benzo-[*c*]phenanthrene; DMBA, 7,12-dimethylbenz[*a*]anthracene; DB[*a,h*]A, dibenz[*a,h*]anthrancene; 3MC, 3-methylcholanthrene; TCPO, 3,3,3-trichloropropylene 1,2-oxide; P450, cytochrome P450; EH, epoxide hydrolase; THF, tetrahydrofuran; HPLC, high-performance liquid chromatography; CSP, chiral stationary phase.

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formed can be directly resolved by chiral chromatography (Weems et al., 1985). Since the stereoheterotopic enzyme−substrate interaction determines the stereochemistry and optical purity of products formed, the enantiomeric composition of the metabolically-formed epoxide is therefore a direct measure of the stereoheterotopic interaction between a cytochrome P450 isozyme and any double bond of a PAH. Epoxide hydrolase catalyzes the *trans* addition of water to an epoxide to form *trans*-dihydrodiol. The enantiomeric pairs of many *trans*-dihydrodiols of PAHs have been separated by chiral stationary-phase HPLC (CSP-HPLC) (Weems et al., 1985; Yang et al., 1989). If the absolute configuration of the epoxide enantiomer is known, regioselective hydration at the *S*- or *R*-carbon of an epoxide enantiomer by epoxide hydrolase (Yang, 1988) can be determined by analyzing the enantiomeric ratio of the resulting dihydrodiol.

Molecular biology techniques have led to the successful cloning and expression of a large number of human P450s (Gonzalez, 1989, 1991; Gonzalez & Korzekwa, 1995). This has permitted the identification of individual P450s responsible for the metabolism of many endobiotics and xenobiotics (Gonzalez et al., 1991; Guengerich & Shimada, 1991). Although stereoselective metabolism of a variety of PAHs was extensively characterized during the past 15 years, liver microsomes of rats treated with specific inducers as enzyme sources were usually employed for accomplishing these studies. Since the multiplicity of P450s constitutively contained in rat liver and induction of the multiple P450 enzymes by chemical inducers, overlapping specificity of these enzymes and an imbalance in the amounts between enzymes induced in liver microsomes may be involved. For example, phenobarbital induces 2B1, 2B2, and 3A1 (Guzelian et al., 1988; Okey, 1990) and 3-methylcholanthrene (3MC) induces 1A1 and 1A2 (Thomas et al, 1987), and both induce many phase II conjugating enzymes (Okey, 1990). Expression of a single P450 protein from a recombinant vector system allows the precise characterization of individual P450s responsible for the metabolism of a particular substrate. In this report, we utilized cDNA-expressed human and mouse 1A1 and 1A2 in the presence and absence of epoxide hydrolase to examine the stereoselective K-region metabolism of B[*a*]P, benz[*a*]anthracene (BA), 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA), dibenz[*a,h*]anthracene (DB[*a,h*]A), benzo[*c*]phenanthrene (B[*c*]Ph), and chrysene (CR). The results provide a basis for understanding the stereochemical course of enzyme-catalyzed reactions of these PAH substrates.

## MATERIALS AND METHODS

*Materials*. (±)B[*a*]P 4,5-epoxide, (±)B[*a*]P 4,5-dihydrodiol, (±)B[*a*]P 9,10-dihydrodiol, (±)3MC 11,12-epoxide, (±)3MC 11,12-dihydrodiol, (±)CR 5,6-epoxide, (±)CR 5,6-dihydrodiol, (±)DB[*a,h*]A 5,6-epoxide, (±)DB[*a,h*]A 5,6-dihydrodiol, (±)DMBA 5,6-epoxide, (±)DMBA 5,6-dihydrodiol, (±) B[*c*]Ph 5,6-epoxide, and B[*c*]Ph 5,6-dihydrodiol were purchased from National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO). TCPO and triethylamine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Construction and expression of recombinant baculoviruses encoding human P450 1A1 as well as enzyme purification were previously described (Buters et al., 1995). Human TK$^-$ 143 cells (thymidine kinase-deficient human embryoblast) containing vaccinia viruses encoding cDNAs of human 1A2, mouse 1A1 and 1A2, and human epoxide hydrolase as stocks were prepared as previously reported (Balltula et al., 1987; Gonzalez et al., 1991; Chakrabarti et al., 1985). Human P450 oxidoreductase was obtained from Dr. James Hardwick (Northeastern Ohio University College of Medicine). All Pirkle-concept chiral stationary-phase columns were purchased from REGIS Technologies, Inc. (Morton Grove, IL)

*Expression of Cytochrome P450s from Recombinant Baculovirus and Vaccinia Viruses*. Insect SF9 cells were infected with recombinant baculoviruses to express human P450 1A1 (Buters et al., 1995). Catalytic activities of P450 enzymes for B[*a*]P metabolism were measured by reconstitution with lipid, P450 oxidoreductase, and other factors and were optimized with the proper ratio of P450 oxidoreductase to P450 1A1. Expression of human 1A2 and mouse 1A1 and 1A2 in hepatoma G2 cells (HepG2) infected with recombinant vaccinia viruses containing cDNAs were previously described (Shou et al., 1994a). HepG2 cells, grown to more than 90% confluence on 175 cm$^2$ plastic flasks, were infected with vaccinia viruses. The cells were harvested 24 h after infection, and their P450 contents were measured by $Fe^{2+}$-CO versus $Fe^{2+}$ difference spectroscopy (Omura & Sato, 1964). Human epoxide hydrolase was expressed from TK$^-$ 143 cells. For metabolism assays, the cells were sonicated and centrifuged at 500000*g*, and pellets containing the membrane proteins were resuspended in 50 mM potassium phosphate buffer ($KP_i$, pH 7.5).

*Stereoselective Metabolic Formation of Epoxides*. Enzymatically formed K-region epoxide was isolated from a mixture of products formed by incubation of the BA, DMBA, CR, B[*a*]P, DB[*a,h*]A, or B[*c*]Ph with individual P450s. Each 10-mL incubation contained 2 nmol of the P450, 50 mM $KP_i$ (pH 7.4), 6 nmol of the epoxide hydrolase inhibitor, TCPO, and 100 mM each of six PAH substrates. The reaction mixture was preincubated at 37 °C for 2 min in a water shaker bath, 1 mM NADPH was then added, and the mixture was incubated for an additional 20 min. Since human P450 1A1 was obtained from membrane fractions of SF9 cells, the mixture of the individual PAHs with human 1A1 was accomplished by reconstitution of 1 nmol of 1A1, 100 mg of dilauroylphosphatidylcholine, 5 nmol of human P450 oxidoreductase, and other factors listed above. Residual PAH substrate and its metabolites were extracted by addition of 6 vol of DCM containing 0.5% triethylamine. The organic solvent extracts were vortexed, centrifuged, and evaporated at 50 °C to dryness under a stream of nitrogen. The K-region epoxide-containing residue formed in the metabolism of each parent PAH was redissolved and applied to normal-phase HPLC for epoxide isolation as described below.

*Metabolic Formation of trans-Dihydrodiol Enantiomers*. Incubation of the PAH substrates with the cDNA-expressed P450s, respectively were carried out except for adding TCPO as described above. Since HepG2 cells contain sufficient epoxide hydrolase for conversion of epoxides formed to *trans*-dihydrodiol (Grant et al., 1988; Shou et al., 1994a), supplementary epoxide hydrolase added to reaction mixture was not necessary. Baculovirus-expressed P450 1A1 in SF9 cells was reconstituted with human epoxide hydrolase expressed in TK$^-$ cells. To optimize the amounts of epoxide hydrolase added to the incubation reaction for obtaining

maximal conversion of the epoxide formed to a dihydrodiol, the ratio of human 1A1 to epoxide hydrolase was observed in obtaining the high B[*a*]P 7,8-diol and low 7-phenol formed in the metabolism of B[*a*]P. Thus 0.1 mg of membrane protein of $TK^-$ cells containing human cDNA-expressed epoxide hydrolase in a 1-mL incubation mixture was chosen for the reconstitution with 1A1-catalyzed reaction.

*High-Performance Liquid Chromatography*. HPLC was performed on a Hewlett Packard model HP1050 liquid chromatograph equipped with an HP model 1050 autosampler, a ternary solvent delivery system, and a multiple wavelength detector. The characteristic UV−visible absorption spectra of individual metabolites (epoxides or dihydrodiols) isolated on either normal-phase or reversed-phase HPLC were determined by a diode array detector of the HP1050 series.

*Normal-Phase HPLC*. In the normal-phase operation, epoxide metabolites formed in the metabolism of the PAH substrates by the P450s in presence of TCPO were analyzed by using a DuPont Zorbax SIL column (4.6 mm i.d. × 25 cm). The column was eluted by a linear gradient with a mixture of a varying ratio of ethyl acetate to hexane containing 0.25% (v/v) triethylamine at 1 mL/min. The K-region epoxide formed was identified by comparing the retention time on HPLC and UV−visible absorption spectrum of the epoxide standard, collected by repetitive chromatography, dried under a stream of nitrogen gas, and applied to CSP columns for enantiomeric resolution as seen below.

*Reverse-Phase HPLC*. The PAH *trans*-dihydrodiol metabolites formed were separated on either a DuPont Zorbax ODS (monomeric $C_{18}$) column (4.6 mm i.d. × 25 cm) or a Waters Associates RCM-100 radial compression module fitted with a Nova-Pak $C_{18}$ cartridge (8 mm i.d. x 10 cm; 4 $\mu$m particles). The columns were eluted with a linear gradient from methanol/water (1:1, v/v) to methanol at a flow rate of 1 mL/min. The K-region dihydrodiol formed in the metabolism of each substrate was identified by comparing its retention time on HPLC and UV−visible absorption spectrum with the standard.

*Chiral Stationary-Phase HPLC*. The following CSP columns (4.6 mm i.d. × 25 cm, Regis Chemical Co.) were employed for the separation of enantiomers of PAH epoxides and dihydrodiols: (*R*,*R*)-Whelk-O1 column, 4-(3,5-dinitrobenzamido)tetrahydrophenanthrene covalently bound to 5 $\mu$ silica; (*R*,*R*)-phenylglycine column [(*R*)-DNBPG], 3,5-dinitrobenzoyl derivative of phenylglycine bound either covalently or ionically to 5 $\mu$ silica; L-leucine column [(*S*)-DNBL], 3,5-dinitrobenzoyl derivative of leucine bound either covalently or ionically to 5 $\mu$ silica. The elution solvent was 10−30% ethanol/methanol (v:v = 1:1) in hexane depending on the epoxide or dihydrodiol employed, and the flow rate was 1 mL/min.

*Determination of Absolute Configuration of Epoxide and Dihydrodiol Enantiomers.* Since a pair of enantiomers has different elution orders on the specific CSP column [(S)-DNBL or (*R*)-DNBPG], absolute configurations of enantiomeric K-region epoxides (Weems et al., 1985; Yang et al., 1990) and dihydrodiols (Yang et al., 1989, 1990) of the PAHs were readily established according to previous reports. In this study, the CSP-Whelk-O1 column was used to obtain a complete base line separation (Figure 1) and to measure the enantiomeric ratio of either an epoxide or dihydrodiol formed in the stereoselective metabolism of a given PAH.

## RESULTS AND DISCUSSION

The K-region epoxide formed in the metabolism of BA, DMBA, CR, B[*a*]P, DB[*a*,*h*]A, or B[*c*]Ph by cDNA-expressed human and mouse 1A1 and 1A2 in the presence of the epoxide hydrolase inhibitor TCPO was isolated from a mixture of metabolites by normal-phase HPLC according to previously published procedures (Mushtaq et al., 1986, 1989; Yang & Fu, 1984; Yang et al., 1985b, 1987b,c). The K-region *trans*-dihydrodiol formed in the metabolism of each of the PAHs with individual P450s in the absence of TCPO was isolated by reverse-phase HPLC as previously reported (Chiu et al., 1983; Weems et al., 1986b; Yang et al., 1984; Mushtaq et al., 1987; 1989). The identification of metabolically-formed epoxides and *trans*-dihydrodiols was based on their retention times on ordinary-phase HPLC, CSP-HPLC, and UV−visible absorption spectra identical to the authentic standards. The separation of the epoxide and *trans*-dihydrodiol enantiomers formed were directly resolved on CSP-Whelk-O1 column as seen in Figure 1. The absolute configurations of each pair of enantiomers were determined by CSP-HPLC on which the enantiomers exhibited less and stronger retentions by their specific chiral recognitions, leading to different elution orders (Yang et al., 1989, 1990; Weems et al., 1985). Accordingly, the enantiomeric contents of the epoxide or dihydrodiol with a known stereochemistry were measured under CSP-HPLC chromatographic areas of enantiomeric peaks and was utilized to study the stereoselectivity of individual P450s and epoxide hydrolase.

*Stereoselective Epoxidation by P450s*. Because most double bonds of fully unsaturated PAHs are prochiral, a stereoheterotopic epoxidation reaction catalyzed by a cytochrome P450 isozyme may result in optically active products. In an *in vitro* incubation of a PAH with P450s, addition of an epoxide hydrolase inhibitor such as TCPO prevents the hydration of the metabolically-formed epoxides. If the metabolically-formed epoxides are sufficiently stable, the enantiomeric ratio of the epoxides formed (*S*,*R*:*R*,*S*) can be resolved by CSP-HPLC. Metabolically-formed K-region epoxides produced in the metabolism of the PAHs were more chemically stable than non-K-region epoxides (Yang, 1988). Hence, the solution of separation of a pair of K-region epoxide enantiomers formed in the metabolism of a given PAH will reveal the extent of stereoheterotopic interaction between the P450 isozyme and a particular double bond of the parent PAH substrate in the epoxidation reaction.

Enantiomeric ratios of metabolically-formed epoxides listed in Table 1 indicated that cDNA-expressed human and mouse P450s 1A1 and 1A2 preferentially produced the major K-region *S*,*R*-epoxides of BA (95−98%), DMBA (94−97%), B[*a*]P (91−96%), DB[*a*,*h*]A (94−98%), and B[*c*]Ph (87−92%), suggesting that the P450s have high stereoselectivity toward one of the two stereoheterotopic faces of the K-region double bond, with a notable favoring of the formation of *S*,*R*-epoxides enantiomer over the *R*,*S*-enantiomer. However, the stereoselective preference of the P450s was reversed to form major *R*,*S*-epoxide of CR (74−85%). Of all P450 isoforms, their stereoselectivities were highly conserved and slightly differed in the degree of stereoselectivity between isoforms of human and mouse. These results are consistent with those observed from stereoselective formation of K-region epoxides formed by 3MC-induced rat liver

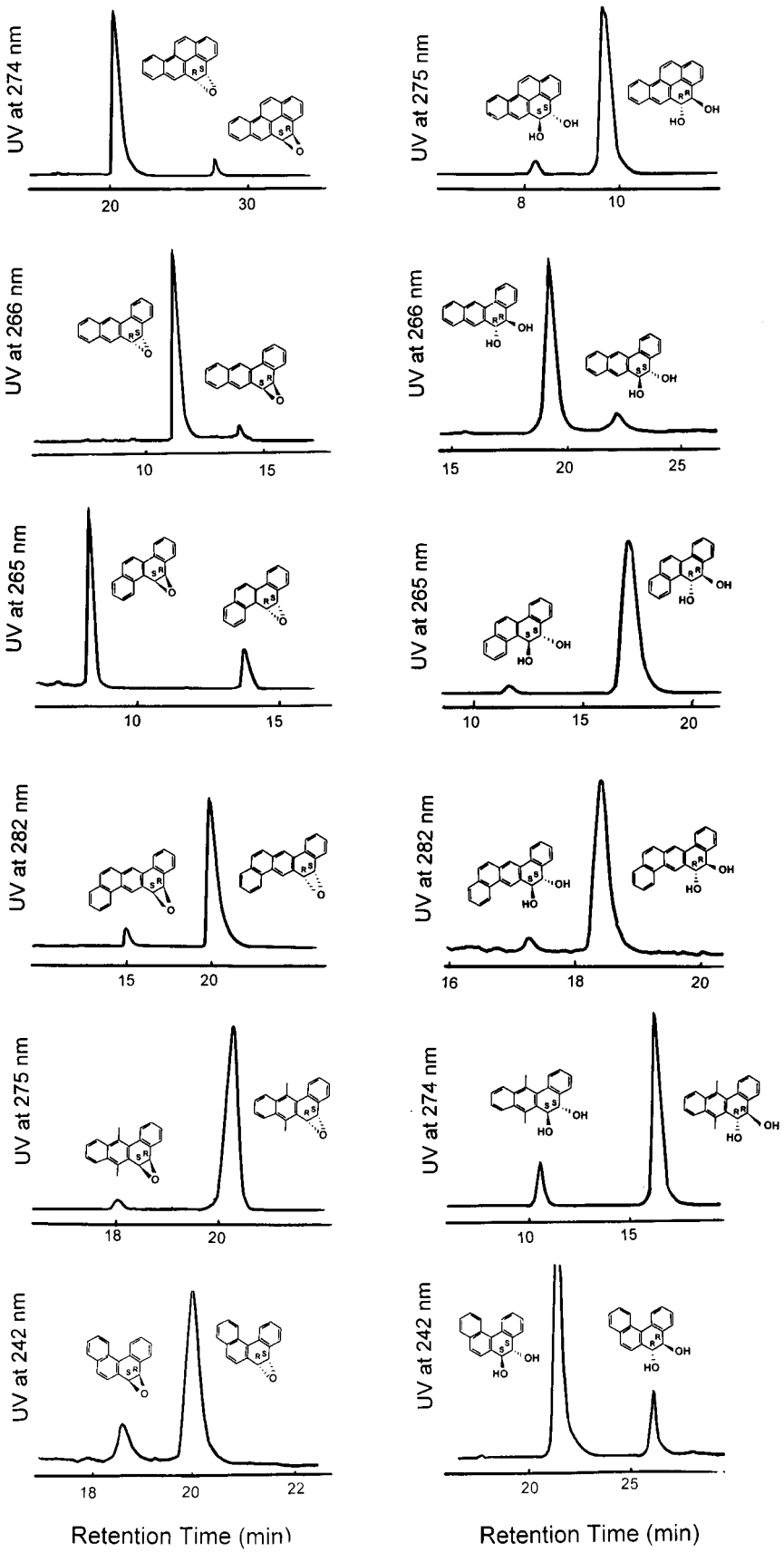


FIGURE 1: CSP-HPLC separation of pairs of epoxide (left side) and dihydrodiol enantiomers formed in the metabolism of B[*a*]P, BA, CR, DB[*a*,*h*]A, DMBA, and B[*c*]Ph by human cytochrome P450 1A1 and epoxide hydrolase. All chiral separations were resolved by a Whelk-O1 column eluted with 10−30% ethanol/methanol (v/v = 1) in hexane at a flow rate of 1 mL/min. Enantiomeric ratios were measured under the chromatographic areas of individual enantiomer metabolites.

microsomes which mainly contain rat 1A1 and 1A2. In this report, 3MC-induced rat liver microsomes predominantly formed K-region *S*,*R*-epoxides of BA (96%) (Yang et al., 1985b), B[*a*]P (96%) (Yang et al., 1985a), B[*c*]Ph (72%) (Yang et al., 1987c), and DB[*a*,*h*]A (99%) (Mushtaq et al., 1989), but similarly their stereoselectivity was reversed to

Table 1: Stereoselective Formation of Epoxides Formed in the Metabolism of Polycyclic Aromatic Hydrocarbons by cDNA-Expressed Human and Mouse 1A1 and 1A2

| | % *S,R*-epoxide:% *R,S*-epoxide[a] | | | | | |
|---|---|---|---|---|---|---|
| P450 | BA-5,6- | CR-5,6- | DMBA 5,6- | B[*a*]P 4,5- | DB[*a,h*]A 5,6- | B[*c*]Ph 5,6- |
| h1A1 | 98:2 | 16:84 | 97:3 | 96:4 | 98:2 | 87:13 |
| h1A2 | 95:5 | 26:74 | 95:5 | 94:6 | 94:6 | 89:11 |
| m1A1 | 95:5 | 15:85 | 97:3 | 95:5 | 97:3 | 92:8 |
| m1A2 | 96:4 | 23:77 | 94:6 | 91:9 | 96:4 | 91:9 |

[a] See Materials and Methods for incubation conditions. Absolute configurations of epoxide enantiomers were determined according to the elution orders of a pair of epoxide enantiomers on CSP-HPLC (Weems et al., 1985; Yang et al., 1990). Enatiomeric ratios of metabolically-formed epoxides were resolved on chiral stationary-phase Whelk-O1 columns as described in the text.

Table 2: Stereoselective Formation of *trans*-Dihydrodiols Formed in the Metabolism of Polycyclic Aromatic Hydrocarbons by cDNA-Expressed Human and Mouse P450s 1A1 and 1A2 in the Presence of Epoxide Hydrolase

| | % (*R,R*-dihydrodiol:*S,S*-dihydrodiol)[a] | | | | | |
|---|---|---|---|---|---|---|
| P450 | BA-5,6- | DMBA 5,6- | CR 5,6- | B[*a*]P 4,5- | DB[*a,h*]A 5,6- | B[*c*]Ph 5,6- |
| h1A1 | 97:3 | 88:12 | 95:5 | 95:5 | 94:6 | 23:77 |
| h1A2 | 95:5 | 80:20 | 93:7 | 91:9 | 92:8 | 12:88 |
| m1A1 | 98:2 | 92:8 | 96:4 | 97:3 | 93:7 | 8:92 |
| m1A2 | 92:8 | 88:12 | 95:5 | 95:5 | 94:6 | 12:88 |

[a] See Materials and Methods for incubation conditions. Absolute configurations of dihydrodiol enantiomers were determined according to the elution orders of a pair of epoxide enantiomers on CSP-HPLC (Yang et al., 1989, 1990). Enatiomeric ratios of metabolically-formed dihydrodiols were resolved on chiral stationary-phase Whelk-O1 columns as described in the text.

yield the major *R,S*-epoxide of CR (95%) (Yang & Bao, 1987b).

The consistent stereochemical outcome for stereoisomeric epoxide formation by all the enzymes in Table 1 suggests that certain structural features of the tertiary structures in the active site of the cytochrome P450s are probably conserved throughout 1A subfamily in different species which determines the steric interaction with substrate and stereoselectivity of epoxidation. Previous studies have shown that nearly identical stereoselectivity of P450s across families were observed in the metabolism of phenanthrene (Shou et al., 1994b) and B[*a*]P (Jones et al., 1995), in which twelve cDNA expressed human and rodent P450s preferentially formed *R,R*-dihydrodiol metabolites. Surprisingly, bacterial $P450_{cam}$ and $P450_{BM3}$ also converted B[*a*]P to the most potent carcinogenic 7*R*,8*R*-dihydrodiol stereoisomer, which was similar to mammalian P450s, although both enzymes exhibited quite low turnovers. Thus, the crystal structures of $P450_{cam}$ and $P450_{BM3}$ provided models by which B[*a*]P was docked in the active sites of the enzymes. Molecular modeling studies suggested that stereoselectivity of P450 reactions is determined by (1) the large size of the substrate molecule and its constraints in the active site, (2) the steric interaction of the substrate with specific amino acids, and (3) a single helical region that is likely to be conserved within the P450 superfamily. Although $P450_{cam}$ and $P450_{BM3}$ have a very low primary sequence homology with the mammalian P450s such as 1A1 and 1A2, at least some features of the tertiary structures of the P450s are likely conserved.

*Regioselective Hydration by EH*. In absence of TCPO, epoxide hydrolase contained in HepG2 cells or added in reconstituted system hydrated a PAH epoxide to *trans*-dihydrodiols. The enantiomers of the K-region dihydrodiol formed in the metabolism of each parent PAH were separated by CSP-HPLC (Figure 1). Their absolute configurations of the dihydrodiol enantiomers were determined by their elution orders on CSP-HPLC (Yang et al., 1989, 1990). Since the stereoselectivity of P450 enzymes in the 1A subfamily resulted in the formation of major enantiomeric epoxides of PAHs, e.g., major *S,R*-epoxides for BA, DMBA, B[*a*]P, DB[*a,h*]A, and B[*c*]Ph, or major *R,S*-epoxide for CR (Table 1), regioselective hydration by the epoxide hydrolase contained in the incubation system mainly determines enantiomeric formation of the resulting *trans*-dihydrodiol. Accordingly, the enantiomeric ratio (*R,R*:*S,S*) of a dihydrodiols formed was utilized to predict the regioselective attack of epoxide hydrolase at one of two chiral centers of the major enantiomeric epoxide as indicated by the arrows in Figure 2. Thus, stereoselective epoxidation by a P450 that preferentially forms one epoxide enantiomer and regioselective hydration of the epoxide enantiomer by epoxide hydrolase which leads to a major dihydrodiol enantiomer will provide a clear understanding of how these enzymes catalyze stereoselective metabolism at the K-region of a PAH.

Among six PAHs, five were metabolized by the four P450 isoforms in the presence of TCPO to form major *S,R*-epoxide enantiomers (87−98%), and one was metabolized to form a major *R,S*-epoxide enantiomer (74−85%), respectively (Table 1). These major epoxide enantiomers formed underwent further hydration by epoxide hydrolase contained either in HepG2 cells or added to a reconstituted system. The enantiomeric ratios of dihydrodiols formed in the metabolism of the PAHs were resolved by CSP-HPLC (Figure 2), and the results in Table 2 indicated that K-region *R,R*-dihydrodiols appeared to be major enantiomers for BA (92−98%), CR (93−96%), DMBA (80−92%), B[*a*]P (91−97%), and DB[*a,h*]A (92−94%). However, the opposite result was observed for B[*c*]Ph metabolism which directed a major *S,S*-dihydrodiol (77−92%).

A pair of dihydrodiol enantiomers with various enantiomeric compositions in the metabolism of a parent PAH is apparently due to the combined result of both stereoselective epoxidation of P450s and regio- and enantioselective hydration of epoxide hydrolase. Although stereoselective epoxidation by the P450 1A isoforms was not exclusive, regioselective hydration of a major epoxide in a pair of epoxide enantiomers by epoxide hydrolase is responsible for the

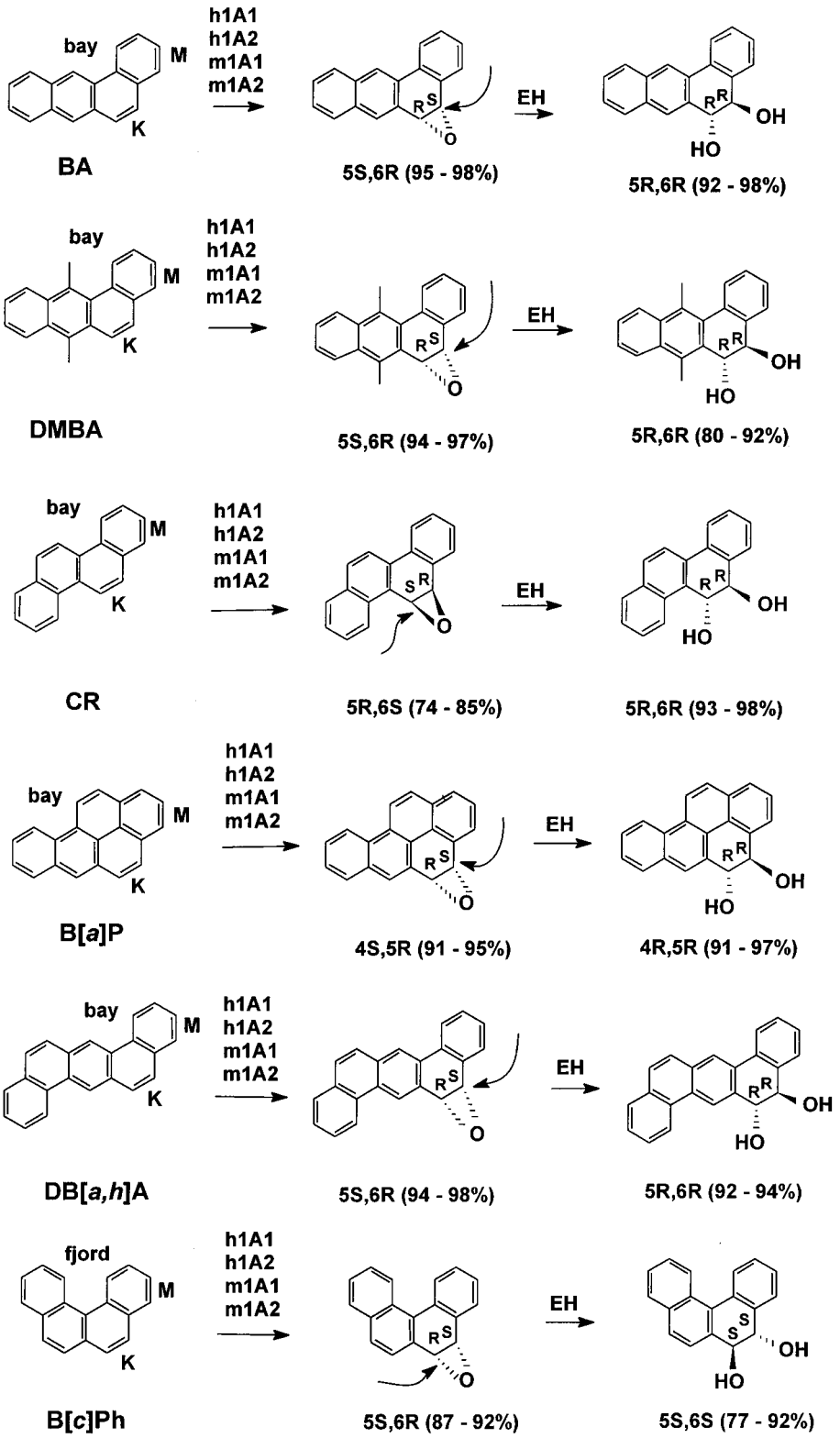


FIGURE 2: Stereoselective metabolic pathways in the epoxidation and hydration at K-region of B[*a*]P, BA, CR, DB[*a*,*h*]A, DMBA, and B[*c*]Ph by human and mouse P450s 1A1 and 1A2 and epoxide hydrolase are proposed. Percentages of K-region epoxide enantiomers formed were resolved by chiral chromatography. Major regioselective attack at either *S*- or *R*-carbon by epoxide hydrolase as shown in arrows was determined by enantiomeric compositions of the resulting dihydrodiols formed in the metabolism of PAHs examined. h1A1 = human P450 1A1; m1A1 = mouse P450 1A1.

stereochemical consequence of the dihydrodiol formed. For example in Figure 1, BA was stereoselectively epoxidized by the human P450 1A1 to almost exclusively form 98% 5*S*,6*R*-epoxide. This almost optically pure epoxide enantiomer was then hydrated by epoxide hydrolase via an attack at the *S*-carbon of the 5*S*,6*R*-epoxide to form 97% *trans*-5*R*,6*R*-dihydrodiol. Epoxide hydrolase must selectively add water with inversion of the configuration at the 5*S*-carbon of the major 5*S*,6*R*-epoxide. Although 2% 5*R*,6*S*-epoxide of BA as a minor opposite enantiomer was also induced, water attack at either 5*R*- or 6*S*-carbon of this epoxide by epoxide hydrolase should not significantly change the

enantiomeric ratio (5*R*,6*R*:5*S*,6*S*) of the resulting dihydrodiol, which was mainly derived from the hydration of the major 5*S*,6*R*-epoxide. Similarly, human P450 1A1 selectively formed major *S*,*R*-epoxides of DMBA (97%), B[*a*]P (96%), and DB[*a*,*h*]A (98%), which were subsequently hydrated at the *S*-carbon of *S*,*R*-epoxide by epoxide hydrolase to 88−95% *R*,*R*-*trans*-dihydrodiols. Contrarily, CR was epoxidized by human 1A1 to form 84% 5*R*,6*S*-epoxide while epoxide hydrolase hydrated the *S*-carbon of 5*R*,6*S*-epoxide to 95% *R*,*R*-dihydrodiol. The % excess of *R*,*R*-dihydrodiol of CR (difference between 95% *R*,*R*-dihydrodiol and 84% 5*R*,6*S*-epoxide) was probably due to a result of the regioselective hydration at the *S*-carbon of the minor opposite 5*S*,6*R*-epoxide enantiomer. Interestingly, epoxide hydrolase had the reverse regioselectivity by attacking *R*-carbon of B[*c*]-Ph 5*S*,6*R*-epoxide, which resulted in the *S*,*S*-dihydrodiol (77−92%) as a major enantiomer.

In general, the enantiomeric compositions of the K-region epoxide formed in the metabolism of each parent hydrocarbon and of the dihydrodiol formed in the hydration of major metabolically-formed epoxide enantiomer are apparently due to stereoselective epoxidation by human P450 1A1/2 and regioselective hydration by epoxide hydrolase.

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