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STEREOSELECTIVITY OF CYTOCHROME P-450 ISOZYMES AND EPOXIDE HYDROLASE IN THE METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract—Enantiomeric compositions of epoxides formed in the metabolism of planar benz[*a*]anthracene (BA), benzo[*a*]pyrene (BaP), and chrysene (CR), and nonplanar benzo[*c*]phenanthrene (BcPh), 12-methylbenz[*a*]anthracene (12-MBA) and 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA) by liver microsomes from untreated, phenobarbital-treated, and 3-methylcholanthrene-treated rats are determined either by direct chiral stationary phase HPLC analysis or by the enantiomeric compositions of metabolically formed *trans*-dihydrodiols. Cytochrome P-450 isozymes contained in various liver microsomal preparations have varying degrees of stereoselectivity in catalyzing the epoxidation reactions at various formal double bonds of the polycyclic aromatic hydrocarbons studied. In general, cytochrome P-450c, the major cytochrome P-450 isozyme contained in liver microsomes from 3-methylcholanthrene-treated rats, has the highest degree of stereoselectivity. Regardless of absolute configuration, non-K-region epoxides are converted to *trans*-dihydrodiols by epoxide hydrolase-catalyzed water attack at the allylic carbon. The *S*-center of K-region *S,R*-epoxide enantiomers derived from planar BA, BaP and CR is the major site of epoxide hydrolase-catalyzed water attack. In contrast, the *R*-center of K-region *S,R*-epoxide enantiomers derived from nonplanar BcPh, 12-MBA and 7,12-DMBA is the major site of epoxide hydrolase-catalyzed water attack. However, the K-region *R,S*-epoxide enantiomers of the six polycyclic aromatic hydrocarbons studied are hydrated by microsomal epoxide hydrolase with varying degrees of regioselectivity. Thus the enantiomeric composition of a metabolically formed dihydrodiol is determined by (i) the stereoselective epoxidation at a formal double bond of a parent hydrocarbon by microsomal cytochrome P-450 isozymes and (ii) the enantioselective and regioselective hydration of the metabolically formed epoxide by microsomal epoxide hydrolase.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants and some are believed to cause cancer induction in man [1, 2]. The roles of cytochrome P-450 isozymes and epoxide hydrolase in the stereoselective metabolic activation of the carcinogen benzo[*a*]pyrene (BaP, Fig. 1) has been well established through studies in the past fifteen years (see reviews in Refs 1 and 2). Microsomal cytochrome P-450 isozymes and epoxide hydrolase are components of the drug-metabolizing enzyme complex which catalyze initial steps in the oxidative metabolism of PAHs.

Cytochrome P-450 isozymes are responsible for catalyzing the formation of the following types of products in the metabolism of PAHs: (i) direct hydroxylation products such as phenols (e.g. 6-hydroxybenzo[*a*]pyrene), cyclic alcohols (e.g. 7-hydroxy-7,8-dihydro-BaP and 1-hydroxy-3-methylcholanthrene) [3–5], and hydroxymethyl derivative of methyl-substituted PAHs, and (ii) epoxidation products such as arene epoxides, phenol-epoxides, vicinal dihydrodiol-epoxide (e.g. BaP 7,8-dihydrodiol-9,10-epoxide [1, 2] in which the epoxy oxygen shares the same benzo-ring with the hydroxyl groups), and non-vicinal dihydrodiol-epoxide (e.g. benz[*a*]anthracene (BA) 3,4-dihydrodiol-8,9-epoxide [6]) in which the epoxy oxygen and hydroxyl groups are on different benzo-rings. These oxygenated metabolites may be further converted to glutathione, glucuronide and sulfate conjugates [1].

Because most double bonds of fully unsaturated PAHs are prochiral, a stereoheterotopic epoxidation reaction catalyzed by a cytochrome P-450 isozyme may result in optically active products (Fig. 2). Achiral products are also formed, and these include monohydroxylated (phenolic) products, quinones, diphenols, epoxides formed at the K-region of symmetric PAHs such as phenanthrene, pyrene and benzo[*e*]pyrene, and hydroxymethyl derivatives of methyl-substituted PAHs.

Microsomal epoxide hydrolase is responsible for converting epoxides to *trans*-dihydrodiols [7]. Due to regioselective and enantioselective properties of epoxide hydrolase, hydration of enantiomeric or racemic epoxides results in *trans*-dihydrodiols with various enantiomeric compositions.

Rapid progress has been made in the last few years in the understanding of the mechanisms of cytochrome P-450-catalyzed stereoselective epoxidation and epoxide hydrolase-catalyzed stereoselective and enantioselective hydration in the metabolism of PAHs. Recent reviews on the enantioselective metabolism of PAHs have appeared [6, 8]. Two factors contribute to recent advances: (i) availability of chiral stationary phase HPLC columns in direct (i.e. without prior derivatization of enantiomers with either an achiral or a chiral reagent) separation of enantiomeric cyclic alcohol, epoxide and dihydrodiol derivatives of PAHs [3, 5, 9–20], and (ii) development of an experimental procedure in

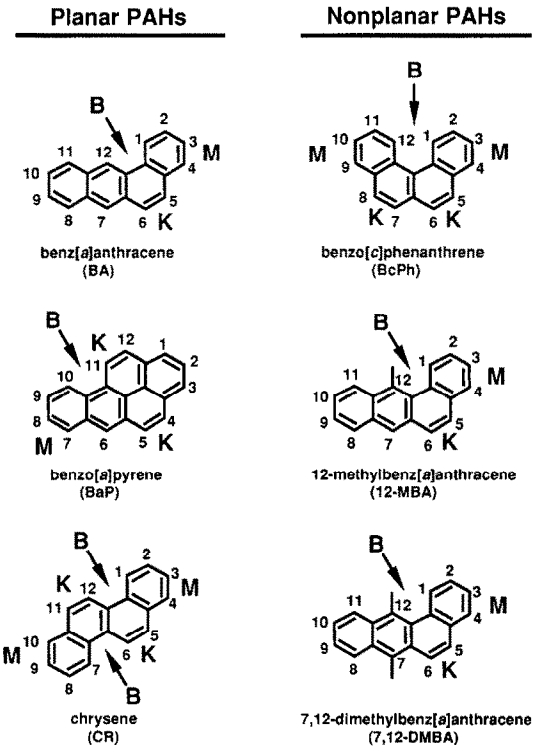

Fig. 1. Structure, numbering, and abbreviation of six polycyclic aromatic hydrocarbons. B, M and K region indicate bay, M and K region, respectively. Bay-region is the sterically hindered region of the molecule. M-region *trans*-dihydrodiol is the metabolic precursor of bay-region dihydrodiol-epoxide. K-region is the most electron-rich region of the molecule. CR is numbered so that the phenanthrene portion of the molecule is oriented in the same manner as the other PAHs.

the stabilization and isolation of metabolically formed K-region and non-K-region epoxides [21–25]. Because K-region epoxides are more stable than non-K-region epoxides, some metabolically formed K-region epoxides have been isolated in earlier studies, although their enantiomeric compositions could not be determined at that time (see Ref. 26 for review). This paper summarizes results obtained in recent studies on the stereoselectivity of rat liver microsomal cytochrome P-450 isozymes and epoxide hydrolase in the enzymatic formation and hydration of enantiomeric epoxides of BA, BaP, chrysene (CR), benzo[*c*]phenanthrene (BcPh), 12-methylbenz[*a*]anthracene (12-MBA) and 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA) (Fig. 1). BA, BaP and CR are planar molecules. BcPh, 12-MBA and 7,12-DMBA are nonplanar molecules due to steric strain in the sterically crowded bay-region. The steric model for the catalytic binding site of cytochrome P-450c originally proposed by Jerina *et al.* [27] is reviewed in light of recent findings.

ABSOLUTE CONFIGURATION OF ENANTIOMERIC DIHYDRODIOLS AND EPOXIDES

Dihydrodiols

The absolute configurations of both K-region and non-K-region dihydrodiol enantiomers can be determined by the exciton chirality circular dichroism (CD) method [28, 29]. Dihydrodiols formed in the metabolism of PAHs are often enriched in one of the two enantiomers. Hence the metabolically formed dihydrodiols can be isolated and used directly in the determination of absolute configurations. Enantiomerically enriched dihydrodiols may also be obtained by chromatographic separation of diastereomers derived from synthetically prepared or biosynthetic dihydrodiols using HPLC columns packed with ordinary stationary phases (e.g. silica or octadecylated silica) [30–32]. Alternatively, an enantiomerically enriched dihydrodiol can be isolated by using HPLC columns packed with one of many chiral stationary phases available [9, 11, 14, 15, 17, 18].

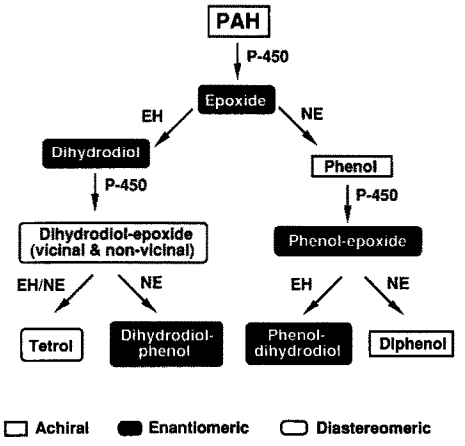

Fig. 2. Oxidative pathways of metabolism of a fully unsaturated PAH leading to chiral (enantiomeric and diastereomeric) and achiral products. Dihydrodiol-phenol and phenol-dihydrodiol are products derived from the metabolism of dihydrodiol and phenol respectively.

The absolute configurations of non-K-region dihydrodiol enantiomers can be determined by the exciton chirality CD method via their tetrahydrodiols. Non-K-region dihydrodiols are readily converted to tetrahydrodiols by a simple catalytic hydrogenation reaction [33]. The absolute configurations of K-region dihydrodiol enantiomers can be determined by the exciton chirality CD method without the need to saturate part of the aromatic system [8, 15, 23, 24, 29, 34].

Non-K-region epoxides

Enantiomers of non-K-region epoxides of tricyclic, tetracyclic, and pentacyclic PAHs are predominantly hydrated by the catalysis of microsomal epoxide hydrolase by water attack at the allylic position to form *trans*-dihydrodiols. These have been established in experiments employing ^{18}O-label using either molecular $^{18}O_2$ or $H_2{}^{18}O$ [6, 8, 22, 25, 35]. Naphthalene 1*S*,2*R*-epoxide is the only non-K-region epoxide known to be hydrated at both allylic and benzylic carbon to form a *trans*-1,2-dihydrodiol with a (1*R*,2*R*):(1*S*,2*S*) enantiomer ratio of 40:60 [36]. Thus the enantiomeric compositions of metabolically

formed non-K-region epoxides derived from tricyclic, tetracyclic and pentacyclic PAHs can be readily estimated by the absolute configuration and enantiomeric composition of the *trans*-dihydrodiols formed. Enantiomers of non-K-region epoxides of some PAHs can be directly separated by chiral stationary phase HPLC [13, 25]. When the absolute configuration of a non-K-region *trans*-dihydrodiol enantiomer is known, information derived from epoxide hydrolase-catalyzed hydration mechanism of the epoxide precursor provides a conclusive result on the enantiomeric composition and absolute configuration of the metabolically formed non-K-region epoxide intermediate.

K-region epoxides

Enantiomers of K-region epoxides of some PAHs can be directly separated by chiral stationary phase HPLC [13, 24]. Reaction of an epoxide with methanol alone or with a methoxide in methanol produced a pair of isomeric monomethyl ethers, which can be readily separated by HPLC [12, 15, 20, 23, 24, 37, 38]. When an enantiomeric epoxide is used, a pair of enantiomeric monomethyl ethers is formed. The absolute configuration of an enantiomeric epoxide can be determined via the determination of the absolute configurations and the location of methyl groups of the two monomethyl derivatives [12, 15, 20, 23, 24, 37, 38]. This approach is much more easily accomplished and provides more reliable results than a previously described method employing ^{18}O-labelling and gas chromatography/mass spectrometry techniques [39].

REGIOSELECTIVE AND STEREOSELECTIVE EPOXIDATION

Epoxides are regioselectively formed in the metabolism of a PAH and its phenolic and dihydrodiol derivatives. The epoxy oxygen may or may not share the same benzo-ring with the hydroxyl group(s) of a phenol-epoxide or a dihydrodiol-epoxide. Due to stereoheterotopic interaction between a substrate and a cytochrome P-450 isozyme, a pair of enantiomers in various enantiomeric compositions may be formed from a parent PAH or its phenolic derivatives. Metabolism of an enantiomeric dihydrodiol can result in two diastereomeric dihydrodiol-epoxides (vicinal and/or non-vicinal) [1, 2, 6, 8, 40]. Diastereomeric vicinal and non-vicinal dihydrodiol-epoxides or their hydration/hydrolysis products (tetrols) can usually be separated on an ordinary stationary phase HPLC column in either the reversed-phase or the normal-phase mode. In this section, only the cytochrome P-450 isozyme-catalyzed stereoselective epoxidation of the parent PAHs will be reviewed. Enzyme-catalyzed stereoselective epoxidation of PAH phenols has so far not been studied. Several reviews are available on the stereoselective metabolism of dihydrodiols to dihydrodiol-epoxides [6, 8, 35, 40].

In an *in vitro* incubation of a PAH with rat liver microsomal enzymes and cofactors, addition of an epoxide hydrolase inhibitor such as 3,3,3-trichloropropylene 1,2-oxide (TCPO) prevents the hydration of the metabolically formed epoxides. The presence of a trace amount of a weak organic base such as triethylamine in the subsequent extraction solvents allowed the isolation of some metabolically formed non-K-region epoxides [22–25]. At the present time, non-K-region epoxides that have been demonstrated as the direct epoxidation products include CR 1,2- and 3,4-epoxides [25], BA 8,9- and 10,11-epoxides [22], BcPh 3,4-epoxide [24] and 12-MBA 8,9- and 10,11-epoxides [23]. Some of these metabolically formed non-K-region epoxides were shown to be optically active [22–24]. Under the experimental conditions described, however, a substantial portion of the metabolically formed non-K-region epoxides was nonenzymatically isomerized to phenolic products. In PAHs studied to date, metabolically formed K-region epoxides can all be isolated with very little or no nonenzymatic isomerization [21–25]. At 0.9 mM of TCPO which does not inhibit the aryl hydrocarbon hydroxylase [41] but completely inhibits the activity of rat liver microsomal epoxide hydrolase, a portion (40–50%, depending on the microsomal preparation used) of cytochrome P-450 isozymes was inactivated (M. Shou and S. K. Yang, unpublished results). However, the effect of TCPO does not appear to be specific for any particular cytochrome P-450 isozyme and does not appear to alter the enantiomeric compositions of metabolically formed K-region and non-K-region epoxides. Inactivation of cytochrome P-450 isozymes by TCPO was reported to be mediated by a cytochrome P-450 isozyme inducible by phenobarbital [42].

Although some non-K-region epoxides can be isolated as metabolites of PAHs [22–25], they are relatively unstable and their enantiomeric compositions cannot be determined by the same technique used for the metabolically formed K-region epoxides (see below). Furthermore, enantiomers of non-K-region epoxides of some PAHs are known to undergo racemization at ambient temperature and in solvents commonly used in *in vitro* metabolism studies [25 and references therein]. However, since all non-K-region epoxides of tricyclic, tetracyclic and pentacyclic PAHs are known to be hydrated by microsomal epoxide hydrolase essentially completely by water attack at the allylic carbon of the epoxide, enantiomeric compositions of metabolically formed non-K-region epoxides can be reliably estimated by the enantiomeric compositions of metabolically formed non-K-region *trans*-dihydrodiols formed (Table 1).

Due to their stability, K-region epoxides formed in the metabolism of a large number of PAHs by various rat liver microsomal preparations in the presence of 0.9 mM of TCPO can be isolated [21–25]. The same procedure should be applicable in general in the *in vitro* metabolism studies of PAHs using other subcellular microsomal enzyme preparations. The enantiomeric compositions of metabolically formed K-region epoxides can be readily determined by HPLC using columns packed with one of several chiral stationary phases [13, 20, 24]. The results obtained by using six PAH substrates and three rat liver microsomal preparations are shown in Table 1. The percentages of *S,R* enantiomers of the K-region epoxides formed in the metabolism of several PAHs by liver microsomes from 3-methylcholanthrene-treated rats are 95–99% for BA, BaP, CR, 12-MBA

Table 1. Enantiomeric compositions of epoxides and *trans*-dihydrodiols formed in the metabolism of several polycyclic aromatic hydrocarbons by rat liver microsomes*

Epoxide or dihydrodiol†	Enantiomer (%)‡					
	S,R-epoxide§			*R,R*-dihydrodiol		
	Control	PB	MC	Control	PB	MC
Benz[*a*]anthracene						
3,4- (M)	~83	~91	~90	83	91	90
5,6- (K)	53,75‖	55,79,69‖	97	77	81	84
8,9-¶	~6	~10	~1	94	90	>99
10,11-	~99	~95	~99	>99	95	>99
Benzo[*a*]pyrene						
4,5- (K)	52	60	95	96	95	>99
7,8- (M)¶	~3	~2	~1	97	98	>99
9,10- (B)	~99	~99	~99	>99	>99	>99
Chrysene						
1,2- (B)¶	~1	~1	~1	99	99	99
3,4- (M)	~51	~41	~96	51	41	96
5,6- (K & B)	32	27	95	86	87	92
Benzo[*c*]phenanthrene						
3,4- (M)	~34	~54	~99**	34	54	>99
5,6- (K)††	60	58	72	17	20	15
12-Methylbenz[*a*]anthracene‡‡						
3,4- (M)	~74	~79	ND§§	74	79	ND
5,6- (K)	73	79	99	7	12	3
8,9-¶	~8	~12	~21	92	88	79
10,11-	~90	~97	~98	90	97	98
7,12-Dimethylbenz[*a*]anthracene‡‡						
3,4- (M)	~57	~62	~64	57	62	64
5,6- (K)	76	80	97	11	5	6
8,9-¶	~9	~4	~1	91	96	99
10,11-	~91	~86	~98	91	86	98

* The data for BaP [8, 21, 50], BA [8, 21, 22, 48], BcPh [24, 51], CR [15, 25], 12-MBA [23] and DMBA [33] were obtained from indicated references.

† Region of the double bond, defined in Fig. 1, is indicated in parentheses.

‡ The sum of two enantiomers is 100%. Control, PB, and MC indicate that liver microsomes were prepared from untreated, phenobarbital-treated and 3-methylcholanthrene-treated rats respectively.

§ Enantiomeric compositions of non-K-region epoxide intermediates are estimated using enantiomeric compositions of dihydrodiols formed. Microsomal epoxide hydrolase-catalyzed hydration of non-K-region epoxides are known to occur essentially completely by water attack at the allylic position [6, 22, 25, 35].

‖ Enantiomeric compositions vary among experiments using rat liver microsomes prepared at different times.

¶ The *R,S*-epoxide enantiomers of these PAHs are the metabolic precursors of *R,R*-dihydrodiols. The positions of CR are numbered as shown in Fig. 1.

** The formation of 3*S*,4*R* enantiomer could not be predicted by the steric model proposed by Jerina *et al.* [27] (see Fig. 7).

†† Our results disagree with those reported by Jerina and coworkers who indicated that BcPh *trans*-5,6-dihydrodiol formed in the metabolism of BcPh by liver microsomes from MC-treated rats was either a racemic mixture [52] or enriched (75%) in the 5*R*,6*R* enantiomer [6].

‡‡ Previously unpublished data on the stereoselective metabolism at the non-K-regions of 12-MBA and 7,12-DMBA will be described in detail elsewhere [53].

§§ ND = not determined.

and 7,12-DMBA, and 72% for BcPh (Table 1). When liver microsomes from untreated and phenobarbital-treated rats were used, the K-region *S,R*-epoxide enantiomer ranges from 52 to 80% respectively for BA, BaP, BcPh, 12-MBA and 7,12-DMBA; 32 and 27% respectively for CR (Table 1). In contrast to some of the non-K-region epoxide enantiomers [25 and references therein], there is no evidence to date indicating that K-region epoxide enantiomers can undergo racemization.

It is clear that 3-methylcholanthrene-inducible cytochrome P-450 isozyme (P-450c), in most but not all cases, has the highest stereoselectivity toward one of the two stereoheterotopic faces of K-region double bonds, favoring the formation of *S,R*-epoxide enantiomer over the *R,S*-epoxide enantiomer (Table 1); the degree of stereoselectivity varies among PAHs. Cytochrome P-450b, the major P-450 isozyme contained in liver microsomes from phenobarbital-treated rats, has a lower preference for the formation of the *S,R*-epoxide enantiomer than cytochrome P-450c. Cytochrome P-450 isozymes contained in liver microsomes from untreated rats, as a whole, have similar stereoselectivity as those in phenobarbital-

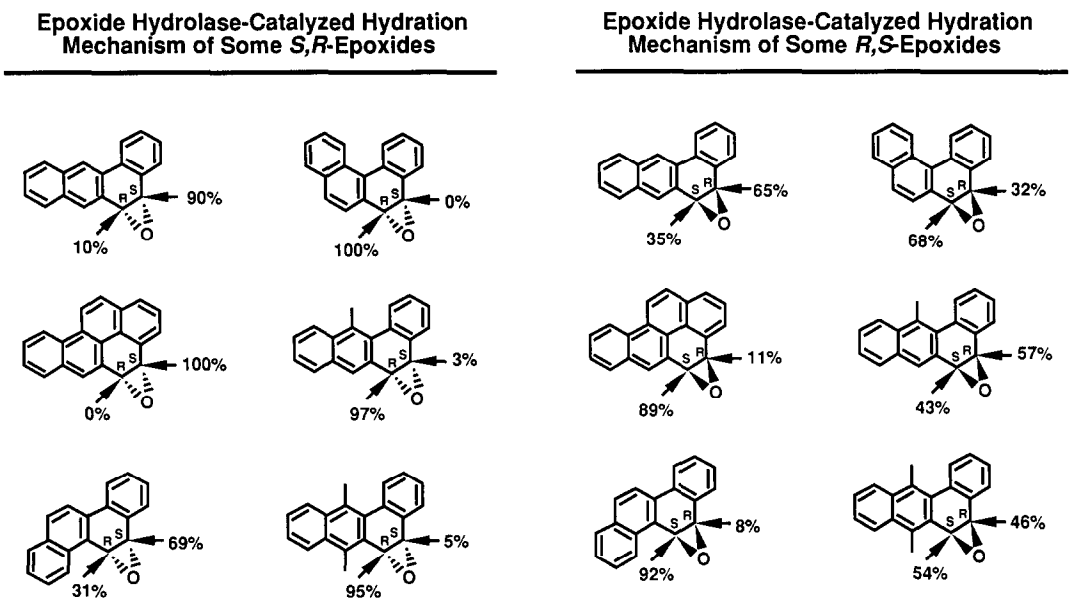

Fig. 3. Mechanisms of microsomal epoxide hydrolase-catalyzed hydration reactions of some enantiomeric K-region epoxides [15, 21, 23, 24]. The percentages of water attack at the *R*- and *S*-centers of the enantiomeric epoxides are indicated by arrows.

treated rats. The most noteworthy observation is that the stereoselective preference for the formation of K-region 5,6-epoxide in the metabolism of CR by cytochrome P-450 isozymes in liver microsomes from 3-methylcholanthrene-treated rats is opposite to those formed by cytochrome P-450 isozymes in liver microsomes from both untreated and phenobarbital-treated rats (Table 1).

REGIOSELECTIVE AND ENANTIOSELECTIVE HYDRATION OF EPOXIDES

Regioselective hydration of an enantiomeric epoxide results in a *trans*-dihydrodiol with various enantiomeric compositions. Epoxide hydrolase may have a higher affinity toward one of two epoxide enantiomers and consequently, enantiomeric epoxides are hydrated with different rates [15, 39]. Thus the hydration reaction is a substrate enantioselective process. Since both enantiomers are often involved in the hydration reaction in PAH metabolism, epoxide hydrolase is said to have enantioselective property. When only one epoxide enantiomer is involved, there is no enantioselective process to speak of and in this case, epoxide hydrolase catalyzes a regioselective hydration reaction. Hence epoxide hydrolase-catalysed hydration reaction is substrate enantioselective but may or may not be product stereospecific. Hydration of non-K-region epoxides is substrate enantioselective and product stereospecific, since epoxide hydrolase generally has a high degree of regioselectivity toward an enantiomeric epoxide [6, 14]. Hydration of K-region epoxides is also substrate enantioselective but in most cases is not product stereospecific (Fig. 3).

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 each epoxide enantiomer (Table 1 and Fig. 3) provide a clear understanding of the reasons why the *R,R*-enantiomer is the major K-region dihydrodiol enantiomer formed in the metabolism of some PAHs while the *S,S*-enantiomer is the major K-region dihydrodiol enantiomer formed in the metabolism of other PAHs. Among six PAHs whose K-region metabolism has been studied in detail, three (BA, BAP and CR) are planar molecules and three (BcPh, 12-MBA and 7,12-DMBA) are nonplanar molecules (Fig. 1). The *S,R*-epoxides (either the 5*S*,6*R* or 4*S*,5*R*) derived from planar PAHs are hydrated by epoxide hydrolase at the *S*-center to form K-region dihydrodiols enriched in the *R,R* enantiomers. The *S,R*-epoxides derived from nonplanar PAHs are hydrated by epoxide hydrolase predominantly at the *R*-center to form the *S,S*-dihydrodiol enantiomers. However, the enantiomeric compositions of K-region dihydrodiols formed by epoxide hydrolase-catalyzed hydration of *R,S*-epoxide enantiomers derived from both planar and nonplanar PAHs vary considerably and there does not seem to be an apparent rule to predict the hydration mechanism of these K-region epoxide enantiomers. Additional studies on the hydration mechanism of K-region epoxide enantiomers of some other planar and nonplanar PAHs are apparently needed before a general rule, if there is one, can be established. The observation that microsomal epoxide hydrolase exhibits a kinetic preference for the addition of water at the oxirane carbon having an *S* absolute configuration of BA 5,6-epoxide and BaP 4,5-epoxide [39] apparently cannot be generalized to include K-region epoxide enantiomers derived from nonplanar PAHs such as BcPh, 12-MBA and 7,12-DMBA (Fig. 3).

CATALYTIC BINDING SITE OF CYTOCHROME P-450 ISOZYMES

With some exceptions (see below), K-region and non-K-region epoxides formed in the metabolism of PAHs by liver microsomes from 3-methylcholanthrene-treated rats are highly enriched in the epoxide (either *S,R* or *R,S*) predicted by the steric model proposed by Jerina *et al.* [27] (Table 1). Because of the high turnover rate of cytochrome P-450c in the metabolism of PAHs, results obtained using liver microsomes from 3-methylcholanthrene-treated rats can be attributed to the major cytochrome P-450 isozyme (P-450c) contained in the microsomes [43]. The K-region epoxides formed in the metabolism of all six PAHs indicated in Table 1 by liver microsomes from untreated and phenobarbital-treated rats contained 27–80% of the *S,R*-enantiomer; the metabolically formed M-region epoxide enantiomers of BA, BcPh, CR, 12-MBA and 7,12-DMBA that are precursors of the *R,R*-dihydrodiols range from 64% to 99%. Thus cytochrome P-450 isozymes contained in liver microsomes from untreated and phenobarbital-treated rats generally exhibit lower stereoselectivity than cytochrome P-450c.

A minimum boundary of a steric model for the catalytic binding site and the position of oxygenation of cytochrome P-450c (Fig. 4) was proposed based initially on the results obtained from metabolism studies of BaP [27]. The results on the enantiomeric compositions of metabolically formed epoxides, either directly determined by CSP-HPLC or indirectly determined via the enantiomeric compositions of the dihydrodiols [6, 27, Table 1 and references therein], formed in the metabolism of several PAHs by either using liver microsomes from 3-methylcholanthrene-treated rats or a reconstituted system containing highly purified cytochrome P-450c can be largely explained by applying the steric model proposed [27]. Enantiomeric compositions of K-region epoxides formed in the metabolism of BcPh and 12-MBA indicate, however, that a substantial amount of 5*R*,6*S*-enantiomer (28%, Table 1) was formed as the result of a binding orientation which is not consistent with the steric model. Although the minimum boundary of the catalytic binding site was developed based initially on the metabolism results of BaP [27], Jerina and coworkers did not consider the occurrence of enzymatic oxygenation at the 2,3-double bond. The 2,3-double bond is a major site of oxygenation by cytochrome P-450c and other isozymes [1, 44]. If the occurrence of oxygenation at the 2,3-double bond of BaP (2*S*,3*R*- and/or 2*R*,3*S*-enantiomer) were considered, the minimum boundary of the catalytic binding site would have been much larger (Fig. 5). In the metabolism of dibenz[*a*,*h*]anthracene (DB[*a*,*h*]A), the 1,2- and 3,4-dihydrodiols constitute 11% and 17% of all the metabolites formed in a reconstituted system containing highly purified cytochrome P-450c and epoxide hydrolase [45]. The 1,2- and 3,4-dihydrodiols formed in the metabolism of DB[*a*,*h*]A by liver microsomes from 3-methylcholanthrene-treated rats have (*R,R*):(*S,S*) enantiomer ratios of 83:17 and 80:20 respectively [6, 29]. However, when 1,2- and 3,4-double bonds, respectively, are each situated at the site of oxygenation, a substantial portion of the DB[*a*,*h*]A molecule is outside of the minimum boundary proposed

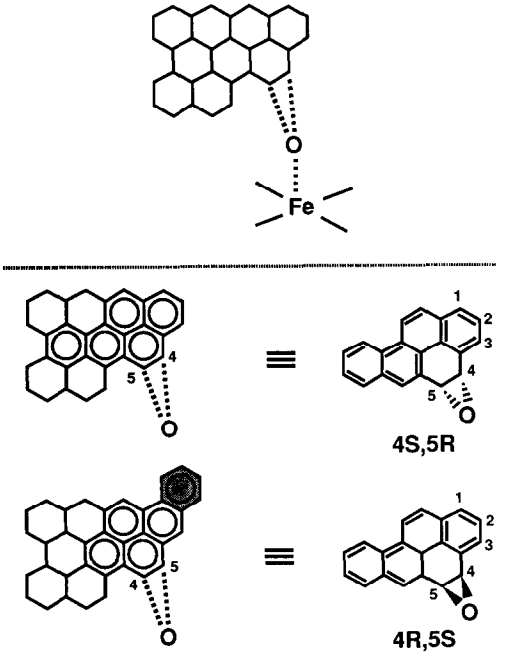

Fig. 4. The steric model for the catalytic binding site and the site of oxygenation of cytochrome P-450c proposed by Jerina *et al.* [27]. The allowed binding mode which yields a 4*S*,5*R*-epoxide and the unfavorable binding mode (with the 7,8,9,10-benzo-ring in the "restricted area" [54]) that may yield a 4*R*,5*S*-epoxide are shown.

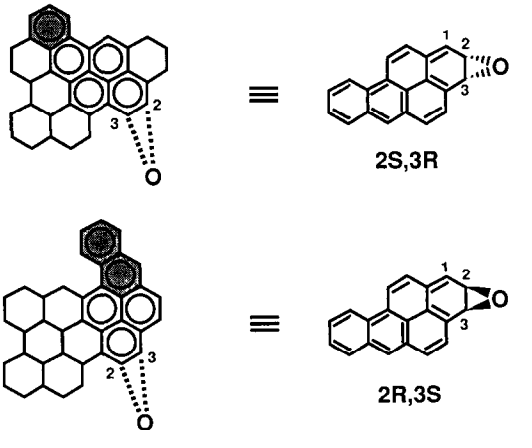

Fig. 5. The unfavorable binding modes for the formations of 2*S*,3*R*-epoxide and 2*R*,3*S*-epoxide in the metabolism of BaP. These unfavorable binding modes have one or two benzo-rings situated in the "restricted area" [27, 54]. Because BaP 2,3-epoxide is unstable and completely isomerized to 3-hydroxy-BaP [44], it is not possible to demonstrate the stereochemistry of the metabolically formed 2,3-epoxide intermediate. The minimum boundary of a steric model is drawn according to Jerina *et al.* [27].

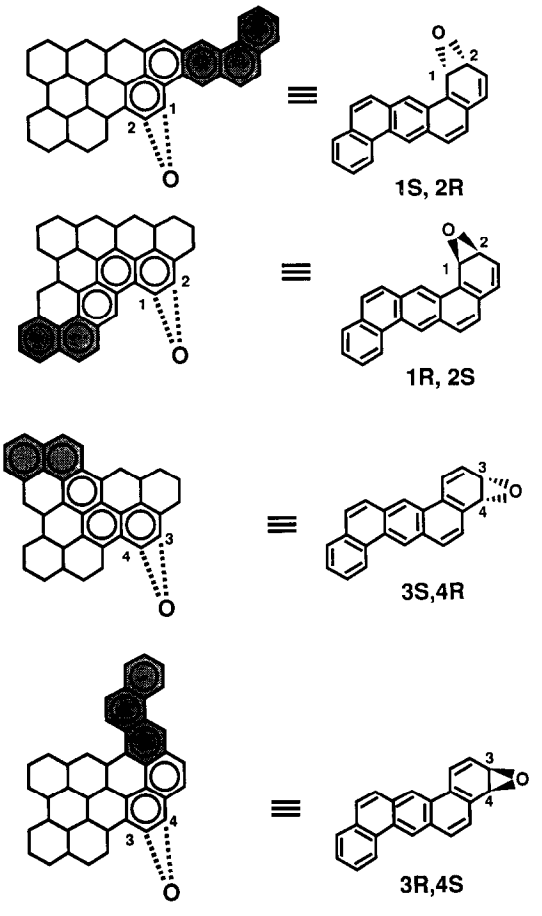

Fig. 6. The unfavorable binding modes for the formations of 1,2-epoxide and 3,4-epoxide in the metabolism of DB[*a,h*]A. These unfavorable binding modes have two or three benzo-rings situated in the "restricted area" [27, 54]. The minimum boundary of a steric model is drawn according to Jerina *et al.* [27].

(Fig. 6). Similarly, the steric model could not be used to predict the enantiomeric composition of BcPh 3,4-dihydrodiol (Fig. 7), 8-methylbenz[*a*]anthracene (8-MBA) 1,2- and 3,4-dihydrodiols [46], and BA 3,4-dihydrodiol [47] formed in the metabolism of BcPh, 8-MBA and BA, respectively. When the K-region 5,6-double bond of DB[*a,h*]A is situated at the site of oxygenation, the whole molecule fits exactly within the minimum boundary of the steric model. However, the 5,6-dihydrodiol is a very minor site of oxygenation by either cytochrome P-450c in a reconstituted system or liver microsomes from 3-methylcholanthrene-treated rats [45]. It is apparent that the steric model [27] can only be used to predict reliably the major epoxide enantiomers formed in the epoxidation of some PAHs, but not all the metabolites (chiral or achiral) of all PAHs. The size of a model for the catalytic binding site of any cytochrome P-450 isozyme should at least be large enough to account for the formation of major metabolites. However, when the size of the model is too large, the model is not useful in predicting the formation of major epoxide enantiomers catalyzed by any cytochrome P-450 isozyme.

Another interesting aspect of the steric model [27] is the authors' speculation that hydroxyl groups of M-region dihydrodiols do not play any role in the metabolic conversion of the M-region dihydrodiol to bay-region dihydrodiol-epoxide [6, 27]. A recent study on the stereoselective metabolism of 7,8-dihydro-BaP [3] was conducted and the results provided additional insight on the possible role(s) of the hydroxyl groups in the metabolic activation of BaP to the ultimate carcinogenic 7,8-dihydrodiol-9,10-epoxide. In the metabolism of BaP 7*R*,8*R*-dihydrodiol, BaP 7*S*,8*S*-dihydrodiol, and 7,8-dihydro-BaP, the 9,10-epoxide stereoisomers (either diastereomer or enantiomer) that were predicted by the steric model (Fig. 8) are ≥95% [6, 35], 86% [6], and 69% [3], respectively (Fig. 9). These results suggest that

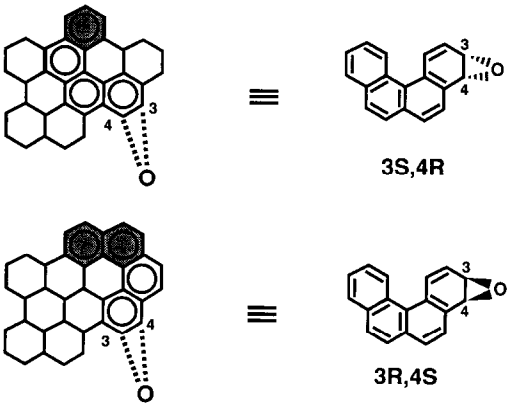

Fig. 7. Possible binding modes for the formations of 3*S*,4*R*-epoxide and 3*R*,4*S*-epoxide in the metabolism of BcPh. Unless the minimum boundary of the proposed catalytic binding site is enlarged, the formation of either 3*S*,4*R*-epoxide or 3*R*,4*S*-epoxide could not be predicted by the steric model proposed [27].

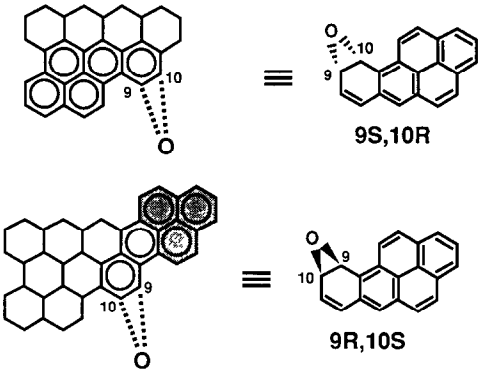

Fig. 8. The binding modes leading to the metabolic formations of 9*S*,10*R*-epoxide (with the entire BaP molecule situated in the "allowed region" [27, 54]) and 9*R*,10*S*-epoxide (with three benzo-rings of BaP situated in the "restricted area" [27, 54]) in the metabolism of BaP. The minimum boundary of a steric model is drawn according to Jerina *et al.* [27]. These binding modes can be similarly used in considering the metabolic formation of 9,10-epoxides in the metabolism of the three 7,8-dihydro derivatives of BaP shown in Fig. 9.

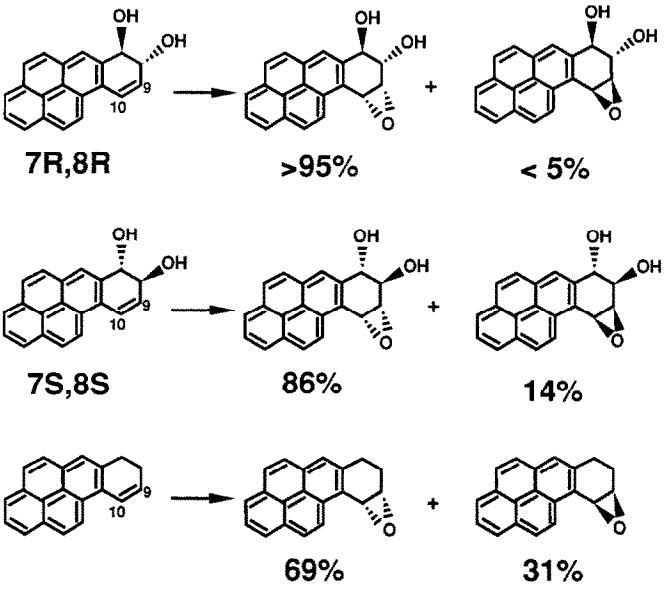

Fig. 9. The percentages of 9,10-epoxide stereoisomers formed in the metabolism of BaP 7*R*,8*R*-dihydrodiol [6, 35], BaP 7*S*,8*S*-dihydrodiol [6], and 7,8-dihydro-BaP [3].

the hydroxyl groups do play an important role in the stereoselective epoxidation of the three 7,8-dihydro derivatives of BaP (Fig. 9). The presence of hydroxyl groups apparently is not ignored by cytochrome P-450c as suggested by Jerina and coworkers [27]. Furthermore, the steric model cannot be used to explain the stereoselective metabolism of the enantiomeric 1,2-dihydrodiols and 3,4-dihydrodiols of BA and DB[*a*,*h*]A [6, 45, 48, 49], unless the minimum boundary is substantially enlarged. At the present time, it is not possible to define the exact size of catalytic binding site in order to account for the observed results in the metabolism studies of PAHs.

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