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Regio- and stereoselectivity of various forms of purified cytochrome *P*-450 in the metabolism of benzo[*a*]pyrene and (−)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene as shown by product formation and binding to DNA

(chemical carcinogenesis/detoxification/hydroxylases/diol-epoxides/benzo[*a*]pyrene activation)

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ABSTRACT Highly purified cytochromes *P*-450$_{LM2}$ and *P*-450$_{LM4}$ and partially purified *P*-450$_{LM1}$, *P*-450$_{LM3b}$, and *P*-450$_{LM7}$ from rabbit liver microsomes exhibit different catalytic activities in the metabolism of benzo[*a*]pyrene (BzP) and (−)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene [(−)*trans*-7,8-diol] in a reconstituted enzyme system. The two highly purified cytochromes also exhibit differences in the activation of BzP and (−)*trans*-7,8-diol to intermediates that bind to DNA, as well as in the stereoselective conversion of (−)*trans*-7,8-diol to the highly mutagenic and carcinogenic diol-epoxides *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol-epoxide I) and *r*-7,*t*-8-dihydroxy-*c*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol-epoxide II). *P*-450$_{LM2}$ is more active than *P*-450$_{LM4}$ in the metabolism of BzP and in its conversion to products that bind to DNA. In contrast, *P*-450$_{LM4}$ is more active than *P*-450$_{LM2}$ in the metabolism of (−)*trans*-7,8-diol and in its conversion to products that bind to DNA. The ratio of activity (percent substrate metabolized) with BzP relative to that with (−)*trans*-7,8-diol is 21 for *P*-450$_{LM2}$ and 0.3 for *P*-450$_{LM4}$; *P*-450$_{LM1}$, *P*-450$_{LM3b}$, and *P*-450$_{LM7}$ gave intermediate ratios. Marked stereoselectivity in the oxygenation of the (−)*trans*-7,8-diol to the highly mutagenic and putatively carcinogenic diol-epoxides I and II was observed with *P*-450$_{LM4}$, whereas the other preparations showed less selectivity. The ratio of diol-epoxide I to diol-epoxide II ranges from 0.3 for *P*-450$_{LM7}$ to 11 for *P*-450$_{LM4}$. The substrate specificity and regio- and stereoselectivity of the different forms of cytochrome *P*-450 may regulate the balance between activation and detoxification pathways of BzP and therefore determine the susceptibility of individual tissues, strains, and species to the carcinogenic action of BzP.

Benzo[*a*]pyrene (BzP), a commonly occurring environmental contaminant which may be involved in human carcinogenesis (1), is itself biologically inactive and requires metabolism by mixed-function oxidases and related enzymes to bind to DNA and exert its toxic (2, 3), mutagenic (4), and transforming effects (4). The mixed-function oxidase systems contain cytochrome *P*-450, which catalyzes the oxygenation of polycyclic aromatic hydrocarbons by a number of pathways, some leading to detoxification and some to the formation of carcinogenic metabolites (2).

As reviewed elsewhere (5), studies in a number of laboratories have been concerned with the question of whether one or more forms of liver microsomal cytochrome *P*-450 (*P*-450$_{LM}$) are responsible for the metabolism of a variety of substrates, including fatty acids, steroids, and prostaglandins, as well as xenobiotics such as drugs, pesticides, and carcinogens. Resolution of the enzyme system into its components, cytochrome *P*-450, NADPH-cytochrome *P*-450 reductase, and phosphatidylcholine (6, 7), was followed by separation and characterization of multiple forms of the cytochrome from rabbit liver microsomes (8). The recent availability of these microsomal enzymes in purified form has made it possible to study polycyclic aromatic hydrocarbon metabolism with the individual catalysts in a reconstituted system (9, 10).

BzP is oxygenated by the mixed-function oxidase system in liver microsomes to form phenols, quinones, and epoxides (11–14), and the epoxide intermediates are further converted to dihydrodiols by the action of epoxide hydrase (15, 16). Most, if not all, of the oxygenated metabolites are converted *in vivo* to water-soluble conjugates. Thus, epoxides are conjugated by glutathione-*S*-transferase (17) and phenols and diols are conjugated by both UDPglucuronate transferase (18) and sulfotransferase (19, 20). One of the pathways of BzP metabolism believed to result in activation involves epoxidation (21–25), followed by epoxide hydrase-catalyzed formation of the (−)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene [(−)*trans*-7,8-diol] (12), which is then further oxygenated by mixed function oxidases to give *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol-epoxide I) and *r*-7,*t*-8-dihydroxy-*c*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol-epoxide II). Thus, the activation of BzP in this process involves three steps, two of which are catalyzed by the mixed function oxidase system. Two isomeric diol-epoxides are formed, the relative amounts of which depend on the stereoselectivity of the second oxygenation step (22, 23). Recent studies have shown that BzP diol-epoxides I and II are very active in binding to DNA *in vitro* and to the RNA and DNA of

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Abbreviations: BzP, benzo[*a*]pyrene; BzP-3-OH, 3-hydroxybenzo[*a*]-pyrene; (−)*trans*-7,8-diol, (−)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene; 9,10-epoxide, benzo[*a*]pyrene 9,10-oxide; triol, trihydroxytetrahydrobenzo[*a*]pyrene; tetrol, tetrahydroxytetrahydrobenzo[*a*]pyrene; diol-epoxide I, *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; diol-epoxide II, *r*-7,*t*-8-dihydroxy-*c*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (in the latter two abbreviations, *r*-7 indicates that the substituent at the 7 position is the reference, and *t* and *c* indicate that the substituents are *trans* and *cis*, respectively, to the reference substituent). (7,10/8,9)-Tetrol indicates that the C_{10}-OH is *cis* and the C_8-OH and C_9-OH are *trans*, respectively, to the reference C_7-OH. Other phenols, diols, triols, and tetrols are similarly designated. *P*-450$_{LM}$, liver microsomal cytochrome *P*-450; the various forms of cytochrome *P*-450 are designated by their relative electrophoretic mobilities when submitted to sodium dodecyl sulfate/polyacrylamide gel electrophoresis, e.g., *P*-450$_{LM2}$.

mammalian cells in culture (24–27), and that diol-epoxide I is more mutagenic than the other BzP derivatives in cultured Chinese hamster V79 cells (22).

In this paper we describe the activities of electrophoretically homogeneous $P\text{-}450_{LM2}$ and $P\text{-}450_{LM4}$ and of other partially purified forms of the cytochrome from rabbit liver microsomes toward BzP and the (−)*trans*-7,8-diol as well as the formation of metabolites that bind covalently to DNA. Evidence is also presented for the regiospecificity of the various forms of $P\text{-}450_{LM}$ toward BzP and the (−)*trans*-7,8-diol and for the stereospecificity of these enzymes in the conversion of the latter compound to diol-epoxides I and II.

MATERIALS AND METHODS

Nonlabeled and 7-^{14}C-labeled (±)*trans*-7,8-diol (specific activity, 25.0 Ci/mol) were obtained through National Cancer Institute Contract NO1-CP33387 (28, 29), and the (−) enantiomers of these compounds were prepared by high-pressure liquid chromatography as described (30). The specific activity of the (−)*trans*-7,8-diol was 11.9 Ci/mol, and that of the [7,*10*-^{14}C]BzP (purchased from Amersham/Searle Corp., Arlington Heights, IL) was 53.9 Ci/mol. BzP was purified prior to use by silica gel column chromatography (31). Due to decomposition upon storage, the radiolabeled (−)*trans*-7,8-diol was purified by high-pressure liquid chromatography (21) just prior to use.

The various forms of cytochrome *P*-450 were isolated from rabbit liver microsomes. $P\text{-}450_{LM2}$ is inducible by phenobarbital but present in only trace amounts in microsomes from untreated or β-naphthoflavone-treated rabbits. $P\text{-}450_{LM4}$ is inducible by β-naphthoflavone and is present at a lower level in the other two types of microsomes. The other forms of $P\text{-}450_{LM}$ are present in all of the microsomal preparations used and are not known to be inducible. Electrophoretically homogeneous $P\text{-}450_{LM2}$ (17.5 nmol/mg of protein) was isolated from phenobarbital-induced microsomes, and the two $P\text{-}450_{LM4}$ preparations (14.0 and 12.9 nmol/mg of protein, respectively) were from phenobarbital- and β-naphthoflavone-induced microsomes (32, 33). Partially purified $P\text{-}450_{LM1}$ and $P\text{-}450_{LM3b}$ (2.2 and 8.8 nmol/mg of protein, respectively) were obtained from phenobarbital-induced microsomes, and $P\text{-}450_{LM7}$ (1.2 nmol/mg of protein) was from microsomes of untreated animals (5, 8). Due to heme loss, the preparations contain varying amounts of apoenzyme, which is not distinguishable from the holoenzyme upon gel electrophoresis. Partially purified NADPH-cytochrome *P*-450 reductase from phenobarbital-induced rabbit liver microsomes (34) had a specific activity toward cytochrome *c* at 30° of 7.4–17.0 μmol reduced per min per mg of protein. The reductase preparations were entirely free of $P\text{-}450_{LM}$. Dilauroylglyceryl-3-phosphorylcholine was obtained from Serdary Research Laboratories, London, ON.

The reaction mixtures, each having a total volume of 1.0 ml, were prepared by adding components in the order shown: Tris·HCl, pH 7.7 (100 μmol), $MgCl_2$ (15 μmol), NADPH (1.13 μmol), sodium deoxycholate (0.25 mg), $P\text{-}450_{LM}$ (0.6 nmol), NADPH-cytochrome *P*-450 reductase (2.8 units), and previously sonicated dilauroylglyceryl-3-phosphorylcholine (60 μg). After 3 min, 20 nmol of [^{14}C]BzP or [^{14}C](−)*trans*-7,8-diol was added in 0.02 ml of methanol or ethanol, respectively, and the incubation was carried out at 30° for 10 or 20 min. The reaction was stopped by the addition of 1.0 ml of acetone at 4°, and the mixture was extracted twice with 2.0 ml of ethyl acetate. Overall product formation was estimated by spectrophotofluorimetry (35). High-pressure liquid chromatography was performed with a Spectro-Physics model 3500 liquid chromatograph on a DuPont Zorbax octadecyltrimethoxysilane column (6.2 mm inner diameter × 0.25 m) with monitoring at 254 nm. The column was eluted at a rate of 0.8 ml/min with a linear gradient from 60% methanol in water to 100% methanol at a sweep time of 50 min for BzP metabolites and 100 min for (−)*trans*-7,8-diol metabolites. Twenty-drop fractions were collected. For the analysis of BzP metabolites, a mixture of synthetic BzP phenols, diols, and quinones was cochromatographed with the sample. A mixture of four BzP tetrols (tetrahydroxytetrahydrobenzo[*a*]pyrenes) and two triols (trihydroxytetrahydrobenzo[*a*]pyrenes) (21) was used as an internal standard for the analysis of the (−)*trans*-7,8-diol metabolites.

DNA binding activity was determined by a modification of published procedures (36, 37). The reaction mixtures, in a total volume of 1.2 ml, contained 0.5 mg of the sodium salt of calf thymus DNA (Sigma Chemical Co.) in addition to the constituents for the metabolic studies as described above and were incubated for 20 min at 30°. After incubation the samples were placed on ice and extracted twice with 3.0 ml of ethyl acetate to remove unbound substrate and metabolites. The aqueous sample was increased to 2.0 ml in volume and extracted with 1.8 ml of phenol reagent (600 g of phenol, 55 ml of water, 75 ml of cresol, and 0.5 g of 8-hydroxyquinoline). The DNA in the aqueous phase was precipitated with three volumes of ethanol (at −20°). The precipitate was dissolved in 1.0 ml of water or 1.0 M sodium acetate/1 mM EDTA, reprecipitated with three volumes of ethanol, and washed with ethanol at 20° and then at 70° for 15 min. The material was hydrolyzed in 0.5 M per-

Table 1. Metabolism of BzP by various forms of cytochrome *P*-450 from rabbit liver microsomes

BzP metabolites formed	Activity of $P\text{-}450_{LM}$ preparations, pmol/min per nmol *P*-450*					
	LM_2	$LM_{4(BNF)}$†	$LM_{4(PB)}$†	LM_1	LM_{3b}	LM_7
trans-9,10-Diol	—	—	2 (3)	195 (44)	—	78 (36)
trans-4,5-Diol	—	—	1 (1)	7 (2)	1 (1)	9 (4)
trans-7,8-Diol	11 (2)	—	0.5 (1)	13 (3)	4 (3)	9 (4)
BzP-9-OH	167 (29)	9 (20)	16 (22)	24 (5)	29 (23)	34 (16)
BzP-3-OH	336 (57)	14 (30)	25 (35)	148 (34)	55 (44)	56 (26)
BzP 1,6-quinone	37 (6)	6 (13)	8 (11)	25 (6)	20 (16)	17 (7)
BzP 3,6-quinone	37 (6)	7 (15)	19 (26)	12 (3)	9 (7)	12 (6)
BzP 6,12-quinone	—	10 (22)	1 (1)	0.3	4 (3)	3 (1)
Others	—	—	—	12 (3)	4 (3)	—
Total	588 (100)	46 (100)	72 (100)	436 (100)	126 (100)	218 (100)

* The numbers in parentheses are the percent of total BzP metabolites. —, No product was detected.

† The $P\text{-}450_{LM4}$ used was isolated from either β-naphthoflavone (BNF)- or phenobarbital (PB)-treated animals, as indicated.

Table 2. Metabolism of (−)*trans*-7,8-diol by various forms of cytochrome *P*-450 from rabbit liver microsomes

Metabolites of (−)*trans*-7,8-diol	Activity of $P\text{-}450_{LM}$ preparations, pmol/min per nmol *P*-450*					
	LM_2	$LM_{4(BNF)}$	$LM_{4(PB)}$	LM_1	LM_{3b}	LM_7
(7,10/8,9)-Tetrol†	3.7 (13)	84.4 (59)	129.6 (59)	19.2 (18)	5.9 (28)	10.9 (6)
(7/8,9,10)-Tetrol†	1.5 (5)	16.8 (12)	28.1 (13)	8.3 (8)	1.3 (6)	2.6 (1)
(7/8,9)-Triol†	0.5 (2)	16.3 (11)	21.1 (10)	1.4 (1)	0.6 (3)	—
(7,9/8,10)-Tetrol‡	2.1 (7)	6.1 (4)	9.9 (5)	7.2 (7)	1.1 (5)	—
(7,9,10/8)-Tetrol‡	4.4 (15)	4.6 (3)	5.8 (2)	10.6 (10)	2.9 (14)	42.8 (24)
Peak *x*	16.5 (58)	16.5 (11)	24.6 (11)	49.8 (48)	9.3 (44)	102.7 (57)
Others	—	—	—	8.8 (8)	—	20.4 (12)
Total	28.7 (100)	144.7 (100)	219.1 (100)	105.3 (100)	21.1 (100)	179.4 (100)

* The numbers in parentheses are the percent of total metabolites extracted into ethyl acetate and estimated by high-pressure liquid chromatography.
† Formed from diol-epoxide I.
‡ Formed from diol-epoxide II.

chloric acid (1.2 ml) at 90° for 15 min, and aliquots were taken for determination of radioactivity and A_{260}. The DNA content was calculated by a modification of the method of Shack (38) in which 40 μg of native calf thymus DNA per ml has an A_{260} of 0.660 after a 22% correction for the hyperchromic effect due to the perchloric acid hydrolysis.

RESULTS AND DISCUSSION

Metabolism of BzP by Various Forms of $P\text{-}450_{LM}$ in the Reconstituted Enzyme System. The metabolites of BzP formed in the reconstituted system containing individual forms of $P\text{-}450_{LM}$ (highly purified $P\text{-}450_{LM2}$ or $P\text{-}450_{LM4}$ from either phenobarbital- or β-naphthoflavone-induced microsomes, or partially purified $P\text{-}450_{LM1}$, $P\text{-}450_{LM3b}$, or $P\text{-}450_{LM7}$) were analyzed by high-pressure liquid chromatography. The separation procedure and the retention times of the BzP metabolites have been reported elsewhere (31). The distribution of the various products and their relative contribution to the total metabolites are shown in Table 1.

Comparison of the total BzP metabolites produced by the different cytochrome preparations shows a much higher activity for $P\text{-}450_{LM2}$ than for $P\text{-}450_{LM4}$ from either phenobarbital- or β-naphthoflavone-treated animals. Similar results were obtained by comparison of the aryl hydrocarbon hydroxylase activities as determined fluorimetrically (unpublished results) and are in agreement with earlier studies (9). A somewhat higher activity was found with $P\text{-}450_{LM4}$ from phenobarbital-induced microsomes than with $P\text{-}450_{LM4}$ from β-naphthoflavone-induced microsomes. It is not clear from these results whether the $P\text{-}450_{LM4}$ preparations from the two sources contain slightly different proteins. These preparations are indistinguishable as judged by immunochemical methods (39), electrophoretic and spectral properties, and the identity of the COOH-terminal amino acids (32).

The BzP-3-OH and BzP-9-OH phenol peaks comprise 86% of the metabolites with $P\text{-}450_{LM2}$ but only about 55% of the metabolites with $P\text{-}450_{LM4}$ from either β-naphthoflavone- or phenobarbital-treated microsomes; the other metabolites are mainly quinones. The relative amounts of the two phenol peaks are different for the two cytochromes, $P\text{-}450_{LM2}$ showing a somewhat greater regioselectivity for the 3 position than does $P\text{-}450_{LM4}$. Since the 1-OH and 7-OH phenols are also contained within the 3-OH peak, it is difficult to determine the specific shift in the position of hydroxylation. A significantly greater proportion of quinones was found in the experiments with $P\text{-}450_{LM4}$ than with $P\text{-}450_{LM2}$. An examination of the amount of dihydrodiols formed indicates that epoxide hydrase is either absent or present at very low levels in the $P\text{-}450_{LM2}$ and $P\text{-}450_{LM4}$ preparations. The metabolism of BzP in the reconstituted system containing partially purified $P\text{-}450_{LM1}$, $P\text{-}450_{LM3b}$, and $P\text{-}450_{LM7}$ was also measured by high-pressure liquid chromatography. $P\text{-}450_{LM1}$ has higher activity than $P\text{-}450_{LM7}$, but the relative distribution of the metabolites is similar. Both enzyme preparations contain epoxide hydrase, as indicated by the large amount of dihydrodiols produced. The *trans*-9,10-diol is produced by both preparations at a very high level in comparison to the other diols, suggesting a high degree of selectivity of $P\text{-}450_{LM1}$ and $P\text{-}450_{LM7}$ for epoxidation at the 9,10-position. On the other hand, the metabolites in the reconstituted system containing $P\text{-}450_{LM3b}$ included only very small amounts of the *trans*-4,5- and *trans*-7,8-diols and no detectable 9,10-diol.

Metabolism of (−)*trans*-7,8-Diol by Various Forms of $P\text{-}450_{LM}$ in the Reconstituted Enzyme System. The relative distribution of the various (−)*trans*-7,8-diol metabolites in the reconstituted enzyme system containing the different forms of $P\text{-}450_{LM}$ is shown in Table 2. Four tetrols and two triols characterized previously (23, 40, 41) were identified by their retention times with high-pressure liquid chromatography. In addition, an unidentified peak (peak *x*) with a retention time of 36 min was observed. The mixed-function oxidases metabolize the (−)*trans*-7,8-diol to diol-epoxide I and diol-epoxide II, which rapidly undergo hydrolysis in the aqueous medium to form specific tetrols and triols. Detection of the specific tetrols and triols indicates which diol-epoxide is formed (21); (7,10/8,9)-tetrol and (7/8,9,10)-tetrol are formed by hydrolysis and (7/8,9)-triol by reduction of diol-epoxide I; (7,9/8,10)-tetrol, (7,9,10/8)-tetrol, and (7,9/8)-triol are derived from diol-epoxide

Table 3. Specificity of various forms of cytochrome *P*-450 from rabbit liver microsomes toward BzP and (−)*trans*-7,8-diol

$P\text{-}450_{LM}$ preparation	% of substrate metabolized*		Activity ratio†
	BzP	(−)*trans*-7,8-Diol	
LM_2	37.7	1.8	20.9
$LM_{4(BNF)}$‡	2.7	8.6	0.3
$LM_{4(PB)}$‡	4.8	14.5	0.3
LM_1	29.7	7.1	4.2
LM_{3b}	8.1	1.3	6.2
LM_7	12.6	10.0	1.3

* For 20-min incubation, determined by high-pressure liquid chromatography.
† Ratio of BzP metabolized to (−)*trans*-7,8-diol metabolized.
‡ $P\text{-}450_{LM4}$ isolated from animals treated with β-naphthoflavone (BNF) or phenobarbital (PB).

Table 4. Stereospecificity of diol-epoxide I and diol-epoxide II formation from (−)*trans*-7,8-diol by various forms by cytochrome *P*-450

Metabolites of (−)*trans*-7,8-diol	Activity, pmol/min per nmol *P*-450*					
	LM_2	$LM_{4(BNF)}$†	$LM_{4(PB)}$†	LM_1	LM_{3b}	LM_7
Diol-epoxide I	5.7 (20)	117.5 (82)	178.8 (82)	28.9 (27)	7.8 (37)	13.5 (7)
Diol-epoxide II	6.5 (22)	10.7 (7)	15.7 (7)	17.8 (17)	4.0 (19)	42.8 (24)
Ratio, I/II	0.9	11.0	11.4	1.6	2.0	0.3

* The numbers in parentheses are the percent of total metabolites extracted into ethyl acetate.
† $P\text{-}450_{LM4}$ isolated from animals treated with β-naphthoflavone (BNF) or phenobarbital (PB).

II (31). The (7,9/8)-triol was not detected in this study, however.

The metabolites formed from the (−)*trans*-7,8-diol in the reconstituted enzyme system were analyzed by high-pressure liquid chromatography as described (21, 31). The catalytic activity of the various forms of $P\text{-}450_{LM}$ toward BzP and the (−)*trans*-7,8-diol is compared in Table 3. The cytochrome preparations show large differences in their ability to metabolize these two substrates; $P\text{-}450_{LM2}$ is about 20 times as active toward BzP as toward the 7,8-diol, whereas the $P\text{-}450_{LM4}$ preparations show a 3-fold greater activity with the diol. The data indicate that $P\text{-}450_{LM4}$ from phenobarbital-treated animals apparently has an even higher total activity toward the diol than does $P\text{-}450_{LM4}$ from β-naphthoflavone-treated animals. $P\text{-}450_{LM1}$ and $P\text{-}450_{LM7}$ are more active than $P\text{-}450_{LM3b}$ in the metabolism of both BzP and the (−)*trans*-7,8-diol.

The distribution of the metabolic products formed from (−)*trans*-7,8-diol indicates that $P\text{-}450_{LM4}$ from both β-naphthoflavone and phenobarbital-treated animals yields primarily diol-epoxide I, whereas $P\text{-}450_{LM2}$ gives diol-epoxides I and II in about equal amounts (Table 4). The $P\text{-}450_{LM1}$ and $P\text{-}450_{LM3b}$ preparations yield chiefly diol-epoxide I, though the ratio of diol epoxides I to II is not as great as that seen with $P\text{-}450_{LM4}$. In contrast, $P\text{-}450_{LM7}$ produces more diol-epoxide II than I. Since these diol-epoxides result from oxygenation on different sides of the ring at the 9,10 position, the results establish the stereoselectivity of the different forms of $P\text{-}450_{LM}$.

The substrate specificity is determined by the form of $P\text{-}450_{LM}$ used, but is also influenced by certain other components in the reaction mixtures. When $MgCl_2$ and sodium deoxycholate were omitted from reaction mixtures containing $P\text{-}450_{LM2}$, the total metabolism of the (−)*trans*-7,8-diol was lower, chiefly due to a decrease in the unknown compound (peak *x*) mentioned earlier.

DNA Binding of BzP and (−)*trans*-7,8-Diol Metabolites Produced by Different Forms of $P\text{-}450_{LM}$. BzP binds to DNA after conversion to the K-region epoxide (4) and the (−)*trans*-7,8-diol after activation to the (−)*trans*-7,8-diol-9,10-epoxide (24, 25). The data presented in Table 5 show that the various forms of $P\text{-}450_{LM}$ convert BzP and the (−)*trans*-7,8-diol to metabolites that bind to DNA to different extents. $P\text{-}450_{LM2}$ has more than twice the activity of $P\text{-}450_{LM4}$ from phenobarbital-treated animals and 4 times the activity of $P\text{-}450_{LM4}$ from β-naphthoflavone-treated animals when radioactive BzP is used as the substrate. In sharp contrast, $P\text{-}450_{LM4}$ from phenobarbital-treated animals has more than 20-fold the activity of $P\text{-}450_{LM2}$ when radioactive (−)*trans*-7,8-diol is the substrate. Again, $P\text{-}450_{LM4}$ from β-naphthoflavone-treated animals appears to be less active than the same cytochrome from phenobarbital-treated animals. The results of such binding experiments are consistent with the metabolic studies on BzP and the (−)*trans*-7,8-diol as described above. The binding studies with the (−)*trans*-7,8-diol are of particular importance since the predominant metabolite bound *in vivo* to DNA (26, 27) and RNA (24) is derived from the diol-epoxide produced from this diol.

Table 5. Binding of BzP and (−)*trans*-7,8-diol metabolites to DNA

$P\text{-}450_{LM}$ preparation	Activity, pmol/mg DNA	
	BzP	(−)*trans*-7,8-Diol
LM_2	9.9 ± 3.2	27.3 ± 4.1
$LM_{4(BNF)}$*	2.5 ± 0.6	429 ± 4
$LM_{4(PB)}$*	4.0 ± 0.2	635 ± 68
Ratio, $LM_{4(BNF)}/LM_2$	0.25	15.7
Ratio, $LM_{4(PB)}/LM_2$	0.4	23.3

* $P\text{-}450_{LM4}$ isolated from animals treated with β-naphthoflavone (BNF) or phenobarbital (PB).

The observed substrate specificity, positional specificity, and stereospecificity of the forms of $P\text{-}450_{LM}$ are of considerable interest, since they may help to explain variations in the susceptibility of different tissues, individuals, and species to the carcinogenic action of polycyclic hydrocarbons. In this connection, it is well established that humans vary in the basal and induced levels of aryl hydrocarbon hydroxylase in both lymphocytes (42) and monocytes (43). The basis of this variation may well be differences in the relative amounts of the individual forms of cytochrome *P*-450. Recent studies have elucidated several of the pathways of BzP activation and detoxication, and have shown that cytochrome *P*-450 plays a key role in both of these processes. The distribution of the various forms of cytochrome *P*-450 in a given tissue or individual may determine the amount of the hydrocarbon converted to a carcinogen relative to the amount detoxified.

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