Stable Expression of Human Cytochrome P450 1B1 in V79 Chinese Hamster Cells and Metabolically Catalyzed DNA Adduct Formation of Dibenzo[*a,l*]pyrene

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Chinese hamster V79 cell lines were constructed for stable expression of human cytochrome P450 1B1 (P450 1B1) in order to study its role in the metabolic activation of chemicals and toxicological consequences. The new V79 cell lines were applied to studies on DNA adduct formation of the polycyclic aromatic hydrocarbon (PAH) dibenzo[*a,l*]pyrene (DB[*a,l*]P). This compound has been found to be an environmental pollutant, and in rodent bioassays it is the most carcinogenic PAH yet discovered. Activation of DB[*a,l*]P in various metabolizing systems occurs via fjord region DB[*a,l*]P-11,12-dihydrodiol 13,14-epoxides (DB[*a,l*]PDE): we found that DB[*a,l*]P is stereoselectively metabolized in human mammary carcinoma MCF-7 cells to the (−)-*anti*- and (+)-*syn*-DB[*a,l*]PDE which both bind extensively to cellular DNA. To follow up this study and to relate specific DNA adducts to activation by individual P450 isoforms, the newly established V79 cells stably expressing human P450 1B1 were compared with those expressing human P450 1A1. DNA adduct formation in both V79 cell lines differed distinctively after incubation with DB[*a,l*]P or its enantiomeric 11,12-dihydrodiols. Human P450 1A1 catalyzed the formation of DB[*a,l*]PDE−DNA adducts as well as several highly polar DNA adducts as yet unidentified. The proportion of these highly polar adducts to DB[*a,l*]PDE adducts was dependent upon both the concentration of DB[*a,l*]P and the time of exposure. In contrast, V79 cells stably expressing human P450 1B1 generated exclusively DB[*a,l*]PDE−DNA adducts. Differences in the total level of DNA binding were also observed. Exposure to 0.1 μM DB-[*a,l*]P for 6 h caused a significantly higher level of DNA adducts in V79 cells stably expressing human P450 1B1 (370 pmol/mg of DNA) compared to those with human P450 1A1 (35 pmol/mg of DNA). A 4-fold higher extent of DNA binding was catalyzed by human P450 1B1 (506 pmol/mg of DNA) compared to human P450 1A1 (130 pmol/mg of DNA) 6 h after treatment with 0.05 μM (−)-(11*R*,12*R*)-dihydrodiol. In cells stably expressing human P450 1B1 the DNA adducts were derived exclusively from the (−)-*anti*-DB[*a,l*]PDE. These results indicate that human P450 1B1 and P450 1A1 differ in their regio- and stereochemical selectivity of activation of DB[*a,l*]P with P450 1B1 forming a higher proportion of the highly carcinogenic (−)-*anti*-(11*R*,12*S*,13*S*,14*R*)-DB[*a,l*]PDE metabolite.

Introduction

Multiple isoforms of cytochrome P450 (P450)[1] play key roles in the metabolic activation of polycyclic aromatic hydrocarbons (PAH) by generating proximate and ultimate carcinogenic metabolites (*1*−*6*). Understanding the role of each of these isoforms is part of a rationale-based risk assessment. Several cellular systems have been developed to study P450-mediated metabolism and me-

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[1] Abbreviations: B[*a*]P, benzo[*a*]pyrene; B[*c*]Ph, benzo[*c*]phenanthrene; DB[*a,h*]A, dibenz[*a,h*]anthracene; DB[*a,l*]P, dibenzo[*a,l*]pyrene; (+)-*syn*-DB[*a,l*]PDE, (+)-*syn*-dibenzo[*a,l*]pyrene-(11*S*,12*R*)-dihydrodiol (13*S*,14*R*)-epoxide; (−)-*anti*-DB[*a,l*]PDE, (−)-*anti*-dibenzo[*a,l*]pyrene-(11*R*,12*S*)-dihydrodiol (13*S*,14*R*)-epoxide; DMBA, 7,12-dimethylbenz-[*a*]anthracene; DMEM, Dulbecco Vogt's modified Eagle medium; EROD, ethoxyresorufin O-deethylation; FITC, fluorescein−isothiocyanate; IgG, immunoglobulins; MC, 3-methylcholanthrene; P450, cytochrome(s) P450; PAGE, polyacrylamide gel electrophoresis; PAH, polycyclic aromatic hydrocarbon(s); PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TBS, Tris-buffered saline; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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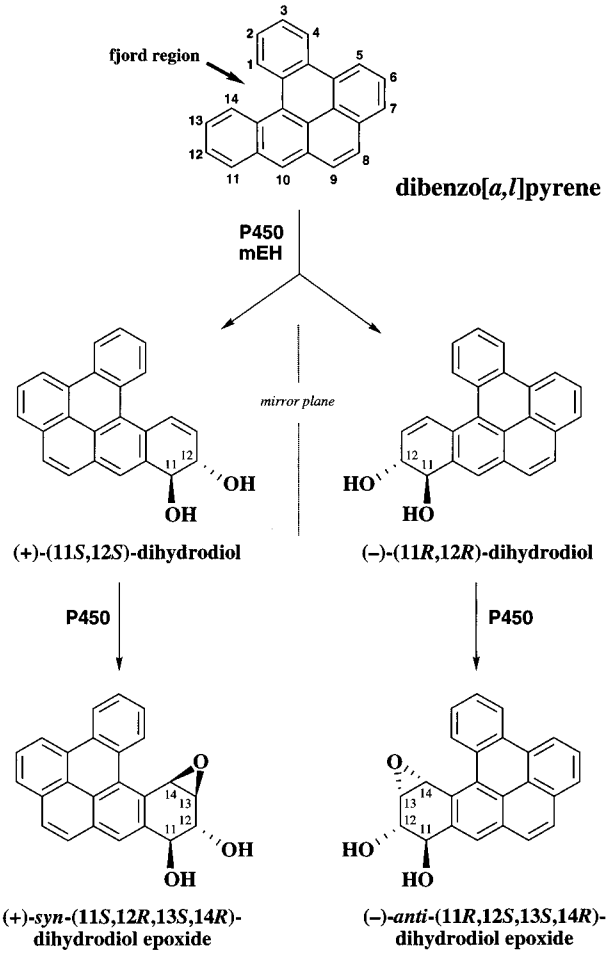
tabolite-dependent toxic effects (*7*). V79 Chinese hamster cells had been selected to serve as host cells for heterologous expression of P450, because these cells possess several biological characteristics which make them a valuable mammalian test system for various toxicological end points (*8*). At the same time, these cells lack detectable P450. Therefore, V79 cells genetically engineered for stable expression of a single P450 isoform allow evaluation of the role of the isoform in metabolite formation and the toxic effect observed under defined experimental conditions.

Recently, a new isoform, P450 1B1, was detected, in mouse first (*9*−*11*) and subsequently in rat (*12*, *13*) and human (*14*, *15*) tissues. P450 1B1 has been found in most extrahepatic human organs including fetal heart, brain, lung, and kidney (*14*, *16*). This isoform is inducible in rodents by exposure to PAH (*9*, *17*), and its importance in the metabolic activation of various members of this group of chemicals has been already indicated by several studies (*16*, *18*−*20*). The metabolic activity of P450 1B1, together with the tissue-specific expression pattern, may help to explain induction of human tumors of extrahepatic origin by procarcinogens activated by this isoform.

Due to its presence in various environmental sources (*21*−*25*) and its exceptionally high carcinogenic activity in rodent bioassays among all PAH tested so far (*26*−*28*), the hexacyclic dibenzo[*a*,*l*]pyrene (DB[*a*,*l*]P) (Scheme 1) must be considered as a potential risk for human health. Studies with mammalian cell cultures (*29*, *30*) and microsomal preparations (*31*−*33*) have demonstrated that this compound is metabolically activated via its 11,-12-dihydrodiols (Scheme 1) to the fjord region *syn*- and *anti*-DB[*a*,*l*]P-11,12-dihydrodiol 13,14-epoxides (*syn*- and *anti*-DB[*a*,*l*]PDE), the most potent mutagenic metabolites of any PAH tested in the Ames assay and Chinese hamster V79 cell system, respectively (*34*). Moreover, both *syn*- and *anti*-DB[*a*,*l*]PDE have been shown to be very potent carcinogens in newborn mice, mouse skin, and rat mammary gland (*35*−*37*).

This exceptional biological activity prompted a number of investigations of DB[*a*,*l*]P. Studies using the human mammary carcinoma MCF-7 cells (*30*) and liver microsomes from rats pretreated with 3-methylcholanthrene (MC) (*31*) demonstrated that metabolic activation of this compound occurs with similar stereoselectivity characteristics as those found for other fjord or bay region PAH such as benzo[*c*]phenanthrene (B[*c*]Ph) or benzo[*a*]pyrene (B[*a*]P). DB[*a*,*l*]P is stereoselectively converted to the (−)-*anti*- and (+)-*syn*-DB[*a*,*l*]PDE with the 11*R*,-12*S*,13*S*,14*R*- and 11*S*,12*R*,13*S*,14*R*-configuration, respectively. In addition, investigations on the metabolic activation of the two immediate precursors of both fjord region DB[*a*,*l*]PDE, the (−)-(11*R*,12*R*)- and (+)-(11*S*,12*S*)-dihydrodiols, respectively (Scheme 1), have revealed very high levels of DNA binding as well as mutagenic activity in V79 cells after metabolic activation of the (−)-dihydrodiol by MCF-7 cells (*38*) or liver preparations from rats pretreated with Aroclor 1254 (*32*). In contrast, the (+)-dihydrodiol bound only weakly to DNA and was not significantly mutagenic. In both test systems the (−)-dihydrodiol was stereoselectively transformed to the (−)-*anti*-(11*R*,12*S*,13*S*,14*R*)-DB[*a*,*l*]PDE in large amounts, whereas corresponding activation of the (+)-dihydrodiol to the (+)-*syn*-(11*S*,12*R*,13*S*,14*R*)-DB[*a*,*l*]PDE was virtually undetectable.

Scheme 1. Stereoselective Bioactivation of Dibenzo[*a*,*l*]pyrene to the Fjord Region 11,12-Dihydrodiol 13,14-Epoxides, Catalyzed by Cytochromes P450 (P450) and Microsomal Epoxide Hydrolase (mEH)[a]



[a] The scheme includes activation to the diastereomeric (+)-*syn*- and (−)-*anti*-11,12-dihydrodiol 13,14-epoxides, which are exclusively formed during metabolism of dibenzo[*a*,*l*]pyrene in MCF-7 cells via the enantiomeric (+)-(11*S*,12*S*)- and (−)-(11*R*,12*R*)-dihydrodiols, respectively (*30*). Arrow indicates the sterically hindered fjord region of the parent compound.

Two crucial steps in the metabolic activation of DB-[*a*,*l*]P to its fjord region DB[*a*,*l*]PDE require oxidation by P450 enzymes (Scheme 1). Human mammary carcinoma MCF-7 cells express P450 1B1 constitutively and P450 1A1 upon induction (*18*). To assess the role of each P450 isoform for metabolic activation of DB[*a*,*l*]P and DNA adduct formation by its metabolites, the cell line V79MZ-h1A1, stably expressing human P450 1A1, and the newly constructed V79MZ-h1B1 cell line, stably expressing human P450 1B1, were exposed to DB[*a*,*l*]P and its enantiomeric 11,12-dihydrodiols and the DNA adducts formed were analyzed.

Experimental Procedures

Caution: *Dibenzo[a,l]pyrene and its enantiomerically pure 11,12-dihydrodiols are mutagenic and carcinogenic and should be handled with care using the guidelines for carcinogenic chemicals of the National Cancer Institute.*

Chemicals. Nuclease P1 (EC 3.1.30.1; from *Penicillium citrinum*), human prostatic acid phosphatase (EC 3.1.3.2; from human semen), apyrase (EC 3.6.1.5; from *Solanum tuberosum*),

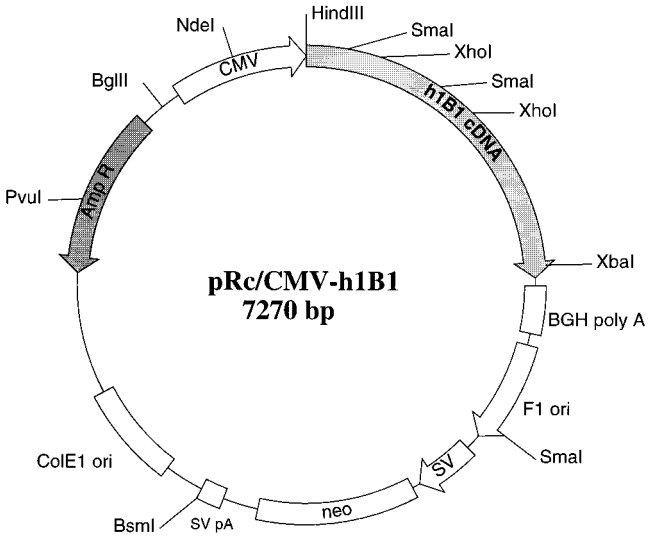


Figure 1. Recombinant eukaryotic expression vector pRC/CMV-h1B1, containing the cytomegalovirus (CMV) promotor attached to the human P450 1B1 cDNA (h1B1) and the selectable marker gene neomycin phosphotransferase (neo).

and phosphodiesterase I (EC 3.1.4.1; from *Crotalus atrox*) were purchased from Sigma Chemical Co. (St. Louis, MO). RNase T1 (EC 3.1.21.3; from *Aspergillus oryzae*) and RNase (DNase-free) were obtained from Boehringer Mannheim Co. (Indianapolis, IN). T4 polynucleotide kinase was obtained from United States Biochemical (Cleveland, OH). [γ-^{33}P]ATP [3500 Ci (129.5 TBq)/mmol] was purchased from Amersham Corp. (Arlington Heights, IL). Proteinase K (EC 3.4.21.64; from *Tritirachium album*) was obtained from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO). Dulbecco's modified Eagle medium, high-glucose type (DMEM with 4.5 g of D-glucose/L), was obtained from Biochrom-Seromed (Berlin, Germany) or Gibco BRL (Grand Island, NY). Antibiotics (penicillin and streptomycin), L-glutamine, and phosphate-buffered saline (PBS without Ca^{2+} or Mg^{2+}; 3.0 mM KCl, 1.5 mM KH_2PO_4, 140 mM NaCl, 8.0 mM Na_2HPO_4, pH 7.0) were purchased from Biochrom-Seromed (Berlin, Germany). Fetal calf serum was obtained from Intergen (Purchase, NY). Antibiotic G418 (Geneticin sulfate) was purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA) or Gibco BRL. Synthesis of DB[*a,l*]P and its enantiomeric 11,12-dihydrodiols was described previously (*32*, *34*, *39*).

Cell Culture. The parental V79MZ cell line (*40*), the genetically engineered cell lines V79MZ-h1A1, expressing human P450 1A1, V79MZ-h3A4, expressing human P450 3A4, V79MZ-r1A1, expressing rat P450 1A1, and V79MZ-r1A2, expressing rat P450 1A2 (*8*), and the newly established V79 cell lines for human P450 1B1 (see paragraph below) were cultivated in Dulbecco's modified Eagle medium (DMEM), high-glucose type, supplemented with 1 mM sodium pyruvate, 4 mM L-glutamine, 10% fetal calf serum, 100 U of penicillin/mL, and 100 μg of streptomycin/mL at 37 °C, 7% CO_2, and 90% saturated atmospheric humidity.

Construction of the pRC/CMV-h1B1 Expression Vector. Human P450 1B1 clone 128 (*14*) was used as the template to create a *Hin*dIII restriction site at the 5′-end and a *Xba*I restriction site at the 3′-end by performing the polymerase chain reaction in order to facilitate insertion of the cDNA into the expression vector pRC/CMV (Invitrogen, Carlsbad, CA). The resulting recombinant expression vector pRC/CMV-h1B1 is shown in Figure 1 with characteristic restriction sites and functions.

Genetical Engineering of V79 Cells for Human P450 1B1. The expression plasmid pRc/CMV-h1B1 linearized with *Pvu*I, and containing the neomycin-phosphotransferase gene as eukaryotic selection marker, was used for transfection of parental V79 cells. For coexpression with human NADPH−P450 reductase, cells were cotransfected with pSV-hCYPOR (*41*, *42*). Transfection was carried out according to the calcium phosphate coprecipitation method of Graham and Van der Eb (*41*, *43*). V79MZ parental cells were transfected with either of the following plasmid preparations: (1) 10 μg of *Pvu*I-linearized pRc/CMV-h1B1 for 1×10^6 cells; (2) 20 μg of *Pvu*I-linearized pRc/CMV-h1B1 for 8×10^5 cells; (3) 1 μg of *Pvu*I-linearized pRc/CMV-h1B1 and 30 μg of pSV-hCYPOR for 8×10^5 cells. Cell clones resistant against 1 mM G418 appeared around 10 days after transfection and were picked the following day. Cell clones were expanded for another 5 days, and aliquots were checked for expression of human P450 1B1 and human NADPH−P450 reductase by in situ immunofluorescence.

In Situ Immunofluorescence. In situ immunofluorescence was performed on tissue culture chambers ("Chamber Slide", Nunc, Naperville) with 1×10^4 cells/well, cultivated for 24 h, washed with PBS, fixed with ice-cold methanol/acetone (1:1, v/v) for 7 min. Fixed cells were incubated with the immunoglobulins (IgG) anti-mouse P450 1B1-IgG from rabbit and anti-human NADPH−P450 reductase-IgG from rabbit (Amersham, Little Chalfont, U.K.) as primary antibodies at a dilution of 1:2000 in DMEM for 1 h at room temperature, followed by three washing cycles with PBS. Fluorescein−isothiocyanate (FITC)-conjugated anti-rabbit IgG from goat (Dianova, Hamburg, Germany) served as secondary antibody. Plates were mounted with 100 mg of *p*-phenylenediamine dihydrochloride (Sigma) in a mixture of 10 mL of PBS and 80 mL of glycerol and covered with a cover slip. Fluorescence was obtained with a fluorescence microscope equipped with standard FITC filter sets (Zeiss Axioplan, Oberkochen, Germany). Selected expressing clones were cultivated for another period of 3 months and periodically checked for expression of human P450 1B1 and human NADPH−P450 reductase by in situ immunofluorescence.

Western Blot Analysis. Cellular homogenates were separated by sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis (PAGE) (8.5% polyacrylamide) according to Laemmli with some modifications (*41*) and transferred onto Immobilon-P protein-binding membrane (Millipore Corp., Bedford, MA). The presence of human P450 1B1 and human NADPH−P450 reductase protein on the Western blots was detected using the same antisera as in the in situ immunofluorescence assay. Finally, the specifically bound rabbit IgG was detected with a horseradish peroxidase-conjugated antiserum from pig (Dakopatts, Hamburg, Germany) by enhanced chemiluminescence (Amersham Int., Little Chalfont, U.K.).

Enzyme Assay. The ethoxyresorufin O-deethylation (EROD) assay was used to measure P450 1B1 or P450 1A1 activity. The enzyme assay was performed with total protein from cells harvested by scraping the cells from the Petri dishes with a rubber policeman. After centrifugation at 1500*g* the cell pellet was shock-frozen in liquid N_2 and stored at −70 °C. The assay was performed as previously described (*44*).

Treatment of V79 Cells with DB[*a,l*]P and DB[*a,l*]P-11,-12-dihydrodiols. Prior to the treatment (24 h) V79 cell lines were seeded at a density of 1×10^6 to 3×10^6 cells/75-cm^2 cell culture flask and grown in a total volume of 30 mL of DMEM, high-glucose type, supplemented with 10% fetal calf serum, and 400 μg/mL G418 at 37 °C, 5% CO_2, and 90% saturated atmospheric humidity. Then 10 μL of a Me_2SO solution of DB-[*a,l*]P or its enantiomerically pure 11,12-dihydrodiols was added.

Cell Harvesting and DNA Isolation. After an incubation period of 6 or 24 h, the supernatant was removed and the cells were harvested by trypsinization with 0.025% trypsin and 0.01% EDTA in PBS. Equal volumes of media supplemented with 10% fetal calf serum were added to stop trypsinization. Subsequently, centrifugation at 2000 rpm and two washing steps with PBS yielded a cell pellet which was stored at −80 °C until DNA isolation. To isolate the DNA from V79 cells, the pellet was resuspended in a solution containing 10 mM Tris, 1 mM EDTA, and 1% (w/v) SDS (pH 8.0), homogenized, and subsequently

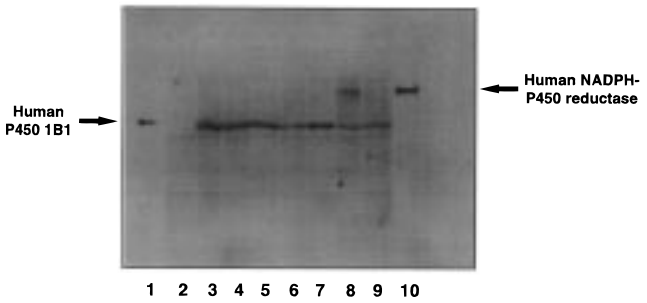


Figure 2. Western blot analysis of the V79 cell clones obtained after individual transfection or cotransfection with plasmid sequences containing the human P450 1B1 and human NADPH−P450 reductase gene, respectively (cf. Table 1). Experimental conditions are described in Experimental Procedures: lane 1, positive control for human P450 1B1; lane 2, parental V79 cell line (*40*); lanes 3−7 and 9, different V79 cell clones (#1−5 and #7) stably expressing human P450 1B1; lane 8, V79 cell clone (#6: V79MZ-h1B1) stably coexpressing human P450 1B1 and human NADPH−P450 reductase; lane 10, positive control for human NADPH−P450 reductase.

incubated for 1 h at 37 °C with RNase T1 (1000 units/mL) and RNase, DNase-free (5 μg/mL), on a shaker (100 rpm). Then proteinase K (500 μg/mL) was added and the incubation continued (1 h at 37 °C). Purification of the DNA was achieved by successive extractions of the mixture with 1 volume of Tris-saturated phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v; three times), followed by separation of the phases by centrifugation at 2000 rpm. Finally, the DNA was precipitated with twice the volume of ethanol, separated, dissolved in water, and stored at −80 °C.

^{33}P-Postlabeling of DNA Adducts. The procedure used was as described previously (*30*). Briefly, 10 μg of DNA isolated from V79 cells after treatment with DB[*a*,*l*]P or its enantiomeric 11,12-dihydrodiols was digested with nuclease P1 and prostatic acid phosphatase, postlabeled with [γ-^{33}P]ATP (3500 Ci/mmol), cleaved to adducted mononucleotides with snake venom phosphodiesterase I, and prepurified with a Sep-Pak C_{18} cartridge (Waters Corp., Milford, MA). Subsequent separation by HPLC (Beckman HPLC system equipped with two 110B pumps and a model 420 controller; Beckman Instruments Inc., St. Louis, MO) was carried out using a C_{18} reverse-phase column (5-μm Ultrasphere ODS, 4.6 × 250 mm; Beckman Instruments Inc.), and the radiolabeled nucleotides were detected by an on-line radioisotope flow-detector (Radiomatic FLO-ONE Beta; Packard Instruments, Downers Grove, IL) as described (*30*). The level of DB[*a*,*l*]P−DNA binding was calculated based on labeling of a [^{3}H]benzo[*a*]pyrene-7,8-dihydrodiol 9,10-epoxide−DNA standard (*45*).

Results

Preparation and Characterization of V79 Cells Stably Expressing Human P450 1B1 and Human NADPH−P450 Reductase. Approximately 50 G418-resistant cell clones were obtained from the first transfection, 400 clones from the second transfection, and 50 clones from the third transfection. After repeated in situ immunofluorescence assays for checking the maintenance of P450 1B1 expression over a 3-month period, seven clones were selected for further investigation: six V79 clones with human P450 1B1 alone and one V79 clone coexpressing human NADPH−P450 reductase. P450 1B1 expression and NADPH−P450 reductase expression in these clones were verified by Western blot analysis (Figure 2), and the enzyme activity in each was verified by EROD activity (Table 1). The cell clone #6 (V79MZ-h1B1) expressing both human P450 1B1 and human NADPH−P450 reductase (Figure 2, Table 1) was selected for use in this study.

Table 1. EROD Activities in Lysates from Parental V79 Cells and Newly Constructed V79 Cell Clones Stably Expressing Human P450 1B1 Alone or Together with Human NADPH−P450 Reductase (cf. Figure 2)[a]

V79 cell clone	EROD (pmol/min/mg)
parental V79 cells	0
V79 cell clone #1	1.83 ± 0.03
V79 cell clone #2	3.59 ± 0.15
V79 cell clone #3	3.15 ± 0.16
V79 cell clone #4	8.06 ± 0.32
V79 cell clone #5	11.5 ± 0.9
V79 cell clone #6[b]	5.11 ± 0.36
V79 cell clone #7	3.24 ± 0.10
V79Mz-h1A1[b]	22.0 ± 0.9
MCF-7 cells[c]	11.3 ± 4.7

[a] EROD data represent the mean of three experiments ± difference of single measurements from the mean. They were calculated from the fluorescence after 30 min of incubation. [b] These clones have been used in the present study. [c] EROD activity was detectable after induction with 10 nM TCDD only (*66*).

DNA Adducts from DB[*a*,*l*]P and Its 11,12-Dihydrodiols in V79 Cells Stably Expressing a Single P450 Isoform. The V79 cell lines stably expressing human P450 1A1, human P450 3A4, rat P450 1A1, and rat P450 1A2, respectively (*8*), in addition to the newly constructed V79MZ-h1B1 cell line, were exposed to DB-[*a*,*l*]P and its 11,12-dihydrodiols in order to measure DNA adduct formation. The DNA was then isolated, postlabeled, and analyzed by HPLC. The HPLC elution profiles of the ^{33}P-postlabeled DNA adducts formed in V79 cells stably expressing human P450 1B1 (V79MZ-h1B1) after treatment with DB[*a*,*l*]P contained four or six peaks, depending on the concentration of the PAH used (Figure 3). Treatment with low doses (0.1 μM) resulted in the formation of four adduct peaks, 1, 2a, 2b, and 4 (Figure 3A), which are formed from (−)-*anti*-DB[*a*,*l*]PDE. These are identical to those identified previously from DB[*a*,*l*]P activated in the human mammary carcinoma cell line MCF-7 [(*30*); that peak numbering system was used to allow direct comparison with results presented in our earlier studies]. At higher concentrations of DB[*a*,*l*]P (1 or 5 μM) two additional peaks (3 and 6) were detected in the adduct profile (Figure 3B). Both are due to covalent interaction of (+)-*syn*-DB[*a*,*l*]PDE with DNA (*30*). At all doses, no DNA adducts eluted with a retention time shorter than 30 min (Figure 3).

In contrast to results with V79 cells stably expressing human P450 1B1, exposure of V79 cells stably expressing human P450 1A1 (V79MZ-h1A1) to DB[*a*,*l*]P resulted in the formation of several DNA adducts with highly polar character that eluted between 5 and 30 min under the HPLC conditions used (Figure 4A,B, polar adducts are labeled with Roman numerals). Treatment of the cells expressing human P450 1A1 with low concentrations of DB[*a*,*l*]P (0.1 μM) resulted in the formation of only these highly polar DNA adducts (Figure 4A). Treatment with higher doses of DB[*a*,*l*]P produced both highly polar adducts and DB[*a*,*l*]PDE−DNA adducts in considerable amounts (Figure 4B). Comparison of the total DNA binding measured in both cell lines after exposure to the lowest concentration (0.1 μM) of DB[*a*,*l*]P revealed a significantly higher level of DNA adducts after metabolic activation by human P450 1B1 irrespective of the time of incubation (Table 2). Levels of 370 pmol of DB[*a*,*l*]-PDE−DNA adducts/mg of DNA were detected in V79MZ-

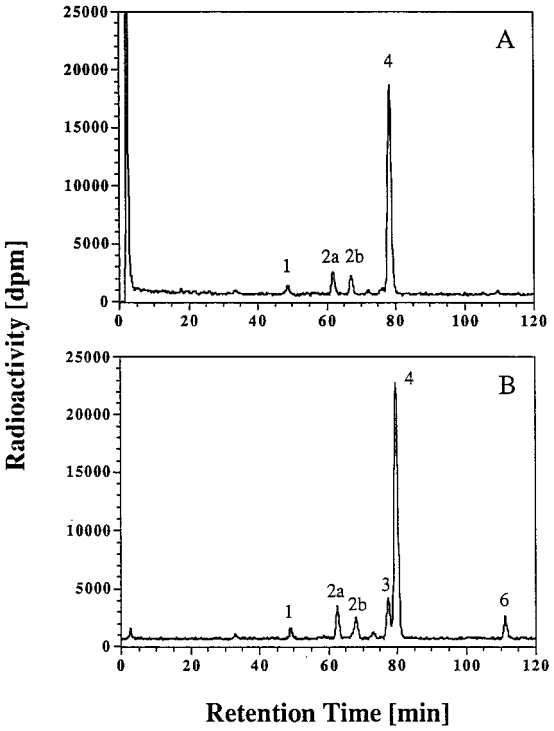


Figure 3. HPLC elution profiles of ^{33}P-labeled DNA adducts of DB[*a*,*l*]P formed in V79 Chinese hamster cells stably expressing the human P450 1B1 enzyme (V79MZ-h1B1) 6 h after treatment with (A) 0.1 μM or (B) 1.0 μM DB[*a*,*l*]P. DNA adducts of the (−)-*anti*-DB[*a*,*l*]PDE (peaks 1, 2a, 2b, and 4) and (+)-*syn*-DB[*a*,*l*]PDE (peaks 3 and 6) were previously identified by cochromatography with authentic standards (*30*). HPLC conditions are described in Experimental Procedures.

h1B1 cells 6 h after treatment with 0.1 μM DB[*a*,*l*]P, whereas only 35 pmol of adducts/mg of DNA (highly polar as well as DB[*a*,*l*]PDE−DNA adducts) were found in V79MZ-h1A1 cells (Table 2). As the dose of DB[*a*,*l*]P increased, both cell lines formed comparable levels of adducts. At the highest dose tested, DNA modification levels were as great as 1 adduct/6000 DNA bases. Interestingly, in the cells expressing human P450 1A1, the percentage of DB[*a*,*l*]PDE−DNA adducts increased from 17% to 87% at 6 h after treatment with increasing concentrations of DB[*a*,*l*]P from 0.1 to 5.0 μM.

To determine whether formation of highly polar DB-[*a*,*l*]P−DNA adducts is also catalyzed by P450 1A1 from other species, V79 cells stably expressing rat P450 1A1 (V79MZ-r1A1) were exposed to DB[*a*,*l*]P. These cells formed highly polar DNA adducts (Figure 4C) similar to those in the cells expressing human P450 1A1 (Figure 4B). However, in comparison to the results obtained with V79MZ-h1A1 cells (Figure 4A,B), the amount and relative proportion of these highly polar adducts were much smaller than those of the DB[*a*,*l*]PDE−DNA adducts (Figure 4C, Table 2). The adduct profiles formed from the cells expressing rat P450 1A1 appeared to look like a composite of the profiles from cells expressing human P450 1B1 and human P450 1A1 (cf. Figures 3, 4). Rat P450 1A1 catalyzed the formation of a higher proportion of DB[*a*,*l*]PDE−DNA adducts irrespective of the concentration of DB[*a*,*l*]P (Table 2).

The ability of these cells to metabolically activate DB-[*a*,*l*]P was further characterized by examining the competence of each cell line for activation of the (−)-(11*R*,12*R*)-dihydrodiol, the immediate metabolic precursor

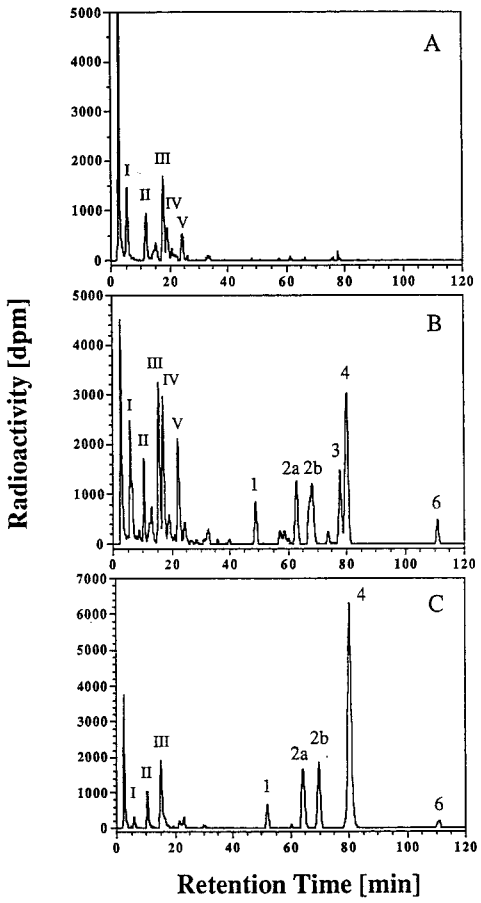


Figure 4. HPLC elution profiles of ^{33}P-labeled DNA adducts of DB[*a*,*l*]P formed in V79 Chinese hamster cells stably expressing the human P450 1A1 enzyme (V79MZ-h1A1) or the rat P450 1A1 enzyme (V79MZ-r1A1): (A) DNA adducts formed in V79MZ-h1A1 cells 6 h after treatment with 0.1 μM DB[*a*,*l*]P; (B) DNA adducts formed in V79MZ-h1A1 cells 6 h after treatment with 1.0 μM DB[*a*,*l*]P; (C) DNA adducts formed in V79MZ-r1A1 cells 6 h after treatment with 0.1 μM DB[*a*,*l*]P. Highly polar unidentified DB[*a*,*l*]P−DNA adducts are labeled with Roman numerals. HPLC conditions are described in Experimental Procedures.

of the (−)-*anti*-(11*R*,12*S*,13*S*,14*R*)-DB[*a*,*l*]PDE (Scheme 1). After treatment of V79MZ-h1B1 cells with different doses of this compound and subsequent ^{33}P-postlabeling, the HPLC adduct patterns obtained consisted exclusively of DNA adducts 1, 2a, 2b, and 4 (Figure 5A), which resulted from formation of (−)-*anti*-(11*R*,12*S*,13*S*,14*S*)-DB[*a*,*l*]PDE (*30*, *38*). As was observed after exposure of these cells to the parent PAH (Figure 4A,B), metabolic activation of the (−)-dihydrodiol by human P450 1A1 resulted in the formation of several DNA adducts with highly polar character (Figure 5B,C). These adducts were the major adducts present after exposure to low concentrations of the (−)-dihydrodiol (0.05 μM, Figure 5B): the proportion of (−)-*anti*-DB[*a*,*l*]PDE−DNA adducts increased considerably after treatment with a high concentration (0.5 μM, Figure 5C). Comparison of the total DNA binding in V79 cells stably expressing human P450 1B1 or human P450 1A1 after exposure to the (−)-dihydrodiol also gave results consistent with those obtained with the parent compound (Table 2). Comparable levels of DNA binding were observed with both P450 isoforms at high concentrations; however, at the low concentration of 0.05 μM the human P450 1B1 isoform was more efficient in catalyzing the formation of DNA

Table 2. Total DNA Binding in V79 Chinese Hamster Cells Stably Expressing One Cytochrome P450 6 or 24 h after Exposure to DB[*a*,*l*]P, (−)-(11*R*,12*R*)-DB[*a*,*l*]P-dihydrodiol, or (+)-(11*S*,12*S*)-DB[*a*,*l*]P-dihydrodiol[a]

	human P450 1B1		human P450 1A1		rat P450 1A1		human P450 3A4	rat P450 1A2
compound (μM)	6 h	24 h	6 h	24 h	6 h	24 h	24 h	24 h
DB[*a*,*l*]P								
0.01					1.5 (1.7)	4.8 (5.0)		
0.1	370	350	6.0 (35)	6.8 (27)	7.9 (9.0)	23 (28)		
1.0	430	280	142 (232)	127 (169)	9.6 (9.6)	140 (145)	0.1	17
5.0	84	433	522 (602)	1380 (1560)	nt	nt		
(−)-(11*R*,12*R*)-dihydrodiol								
0.005	18	22	nt	nt	28 (35)	34 (40)		
0.05	506	1010	31 (130)	49 (175)	252 (310)	393 (490)		
0.5	1650	1680	1560 (1990)	2430 (3000)	929 (1000)	1340 (1350)	6.5	4.0
1.0	nt	nt	2180 (2400)	3250 (3560)	nt	nt		
(+)-(11*S*,12*S*)-dihydrodiol								
0.5	nt	nt	nt	4.4	nt	2.5	1.8	6.0

[a] All data presented are reported in pmol of adducts/mg of DNA. Values represent adducts of DB[*a*,*l*]PDE; values in parentheses represent all adducts found including both those of DB[*a*,*l*]PDE and those of more polar character (see Figures 4, 5). nt, not tested.

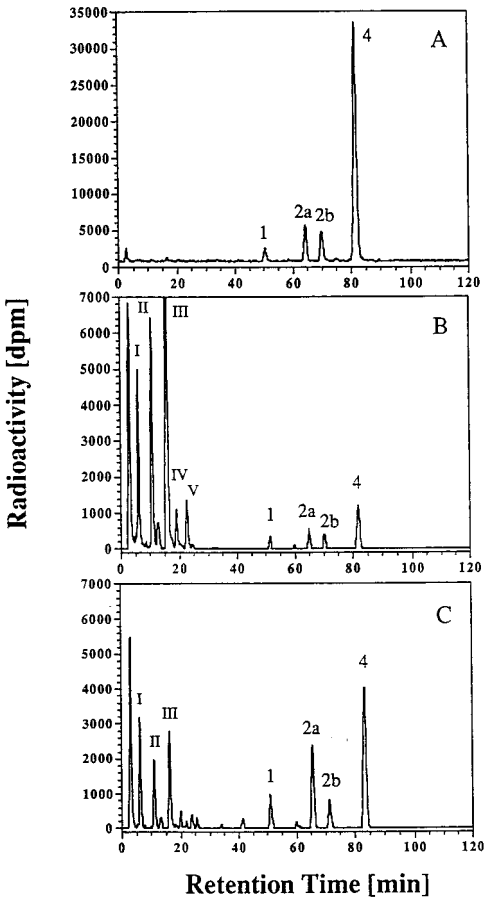


Figure 5. HPLC elution profiles of ^{33}P-labeled DNA adducts of the (−)-(11*R*,12*R*)-dihydrodiol of DB[*a*,*l*]P formed in V79 Chinese hamster cells stably expressing the human P450 1B1 enzyme (V79MZ-h1B1) or the human P450 1A1 enzyme (V79MZ-h1A1): (A) DNA adducts formed in V79MZ-h1B1 cells 6 h after treatment with 0.005 μM (−)-DB[*a*,*l*]P-11,12-dihydrodiol; (B) DNA adducts formed in V79MZ-h1A1 cells 6 h after treatment with 0.05 μM (−)-DB[*a*,*l*]P-11,12-dihydrodiol; (C) DNA adducts formed in V79MZ-h1A1 cells 6 h after treatment with 0.5 μM (−)-DB[*a*,*l*]P-11,12-dihydrodiol. Highly polar unidentified DB[*a*,*l*]P−DNA adducts are labeled with Roman numerals. HPLC conditions are described in Experimental Procedures.

adducts than human P450 1A1 (506 compared to 130 pmol/mg of DNA, respectively). In the cells expressing human P450 1A1 only 24% (31 pmol/mg of DNA) of the total DNA binding was caused by the (−)-*anti*-DB[*a*,*l*]-PDE. At higher concentrations an increasing proportion of DB[*a*,*l*]PDE−DNA adducts (up to 91% after exposure to 1 μM of the dihydrodiol for 6 h) was observed (Table 2). In cells expressing human P450 1B1 only the (−)-*anti*-DB[*a*,*l*]PDE−DNA adducts were detected at all times and concentrations tested (Table 2).

The HPLC adduct patterns obtained after incubation of V79 cells expressing rat P450 1A1 with different doses of the (−)-dihydrodiol contained two major polar adducts and the four (−)-*anti*-DB[*a*,*l*]PDE−DNA adduct peaks (1, 2a, 2b, and 4) which were detected in the human P450-expressing cells (data not shown, cf. Figure 4C). The rat P450 1A1 isoform activated the (−)-dihydrodiol to yield 35 and 310 pmol of adducts/mg of DNA after 6-h exposure to 0.005 and 0.05 μM, respectively. A further increase in the total DNA binding was detected after 24-h incubation (Table 2). At the 0.5 μM dose activation of the (−)-dihydrodiol by the V79 cells expressing rat P450 1A1 was comparable to that observed in cells expressing human P450 1A1 or human P450 1B1 (modification levels in the range of 1 adduct/1000−2000 DNA bases).

Activation of the (+)-(11*S*,12*S*)-dihydrodiol, the precursor of the (+)-*syn*-(11*S*,12*R*,13*S*,14*R*)-DB[*a*,*l*]PDE (Scheme 1), was also examined. Low levels of formation of the (+)-*syn*-DB[*a*,*l*]PDE−DNA adducts were observed in the cells expressing human P450 1B1 and human P450 1A1 after exposure to high doses of DB[*a*,*l*]P (Figures 3B, 4B). In V79 cells stably expressing human or rat P450 1A1, only low levels of the (+)-dihydrodiol were activated to DNA-binding intermediates even at the high concentration of 0.5 μM (Table 2). These P450 1A1 isoforms had much greater ability to activate the enantiomeric (−)-dihydrodiol than the (+)-dihydrodiol (Table 2). However, activation of the (+)-dihydrodiol could contribute to DNA binding for P450 isoforms which possess limited activity for the metabolic activation of parent PAH (e.g., human P450 3A4 or rat P450 1A2). Total DNA adduct levels of 6.5 and 1.8 pmol/mg of DNA could be measured after exposure of V79 cells expressing human P450 3A4 to 0.5 μM (−)-dihydrodiol and its (+)-enantiomer, respectively. In contrast, this isoform activated DB[*a*,*l*]P itself only to a low extent (0.1 pmol of adducts/mg of DNA 24 h after treatment with 1 μM). Cells stably expressing the rat P450 1A2 isoform metabolically activated both enantiomeric dihydrodiols with comparable efficiency: the levels of binding were 6.0 and 4.0 pmol of adducts/mg of DNA after exposure to 0.5 μM (+)- and (−)-dihydrodiol, respectively (Table 2).

Discussion

Various P450 isoforms are responsible for the carcinogenic activity of a large number of PAH by catalyzing the formation of proximate and/or ultimate carcinogenic metabolites (*3*−*6*, *16*). Enzymatic activation of the potent carcinogen DB[*a*,*l*]P to its electrophilically reactive and DNA-binding *syn*- and *anti*-DB[*a*,*l*]PDE requires two epoxidation steps by P450 enzymes (Scheme 1). Measurement of the formation of DB[*a*,*l*]P dihydrodiols by recombinant human P450 1A1 demonstrated that this isoform catalyzes considerable oxidation of DB[*a*,*l*]P at the 8,9-, 11,12-, and 13,14-positions (*5*). The immediate metabolic precursor of the *syn*- and *anti*-DB[*a*,*l*]PDE, the 11,12-dihydrodiol, is also formed by rat liver microsomes of MC-treated animals (*46*) or by lung and liver microsomes of human donors in the age range of 18−60 years (*5*). Moreover, both *syn*- and *anti*-DB[*a*,*l*]PDE−DNA adducts have been detected after incubation of DB-[*a*,*l*]P with liver microsomes of MC-treated (*31*) or Aroclor 1254-treated (*33*) rats in the presence of DNA. Administration of MC or Aroclor 1254 is known to significantly increase the level of P450 1A1 in the liver of the treated rodents (*47*, *48*). All together, these results suggested that P450 1A1 is capable of carrying out both epoxidation steps required for activation of DB[*a*,*l*]P to its fjord region DB[*a*,*l*]PDE (Scheme 1).

Exposure of human mammary carcinoma MCF-7 cells (*29*) or mouse embryo C3H10T1/2CL8 fibroblasts (*49*) to DB[*a*,*l*]P resulted in formation of both *syn*- and *anti*-DB-[*a*,*l*]PDE−DNA adducts. This has also been verified in vivo by analyzing lung tissue of strain A/J mice after ip administration of DB[*a*,*l*]P (*50*). Moreover, it could be demonstrated that this bioactivation is highly stereoselective. MCF-7 cells exclusively generate (+)-*syn*- and (−)-*anti*-DB[*a*,*l*]PDE−DNA adducts which are formed from the respective precursors, the (+)-(11*S*,12*S*)- and (−)-(11*R*,12*R*)-dihydrodiols, respectively (*30*) (cf. Scheme 1). No formation of (+)-*anti*- or (−)-*syn*-DB[*a*,*l*]PDE was detected. Interestingly, human MCF-7 cells express P450 1A1 only upon induction, but P450 1B1 constitutively (*18*). Additional evidence suggesting an important role of human P450 1B1 during biotransformation of PAH was provided by studies of the induction of *umu* gene expression in bacteria after metabolic activation of various dihydrodiols of PAH by purified microsomal P450 1B1 (*16*). The present study was designed to clarify the role of individual human P450 isoforms, especially P450 1B1 and P450 1A1, in the metabolic activation of DB-[*a*,*l*]P.

Chinese hamster V79 cells stably expressing human P450 1A1 were treated with different doses of DB[*a*,*l*]P or its enantiomeric 11,12-dihydrodiols. Comparison to the corresponding turnover catalyzed by human P450 1B1 was feasible using the genetically engineered V79 cell lines stably expressing this P450 isoform. Results obtained from both cell lines are directly comparable. The P450 activities were found to be of the same order of magnitude (cf. Table 1) taking into account that the specific activity of human P450 1A1 for ethoxyresorufin is nearly 7-fold higher than for human P450 1B1 (*51*).

Exposure to different concentrations of DB[*a*,*l*]P revealed that V79 cells stably expressing human P450 1B1 exclusively generate (+)-*syn*- and (−)-*anti*-DB[*a*,*l*]PDE−DNA adducts (Figure 3, Table 2) and thus form exactly the same adducts as previously detected in human MCF-7 cells (*29*, *30*). At low concentrations of DB[*a*,*l*]P (0.1 μM) only (−)-*anti*-DB[*a*,*l*]PDE−DNA adducts could be detected (Figure 3A) indicating either an exclusive generation of the respective *R*,*R*-configurated (−)-dihydrodiol precursor at low doses of the parent compound (cf. Scheme 1) or an increased sequestration of any (+)-*syn*-DB[*a*,*l*]PDE formed by water or other cellular nucleophiles. The latter explanation seems to be more likely in consideration of the finding that *syn*-DB[*a*,*l*]PDE preferentially adopts a conformation with pseudo-diequatorial oriented hydroxy groups (*aligned* conformation) (*34*) and the demonstration that vicinal *syn*-dihydrodiol epoxides preferring this conformation undergo significantly accelerated hydrolytic opening of their oxiranyl ring under neutral conditions compared to corresponding *anti*-diastereomers (*52*, *53*). The dose dependency of the adduct pattern obtained in V79 cells expressing human P450 1B1 (Figure 3A,B) was also observed in MCF-7 cells. (+)-*syn*-DB[*a*,*l*]PDE−DNA adducts were only detected in MCF-7 cells after treatment with high doses of the parent PAH, e.g., 1−8 μM.[2] DNA adducts of (+)-*syn*-DB[*a*,*l*]PDE were virtually undetectable in MCF-7 cells after exposure to 0.05 μM (+)-11,12-dihydrodiol (*38*). These results suggest that nucleophilic sequestration of small amounts of intermediately formed (+)-*syn*-DB[*a*,*l*]-PDE by water or cellular proteins rather than a limited metabolic conversion of DB[*a*,*l*]P to its (+)-11,12-dihydrodiol (cf. above) may account for the absence of (+)-*syn*-DB[*a*,*l*]PDE−DNA adducts in cells treated with small amounts of DB[*a*,*l*]P.

Treatment of V79 cells expressing human P450 1A1 with DB[*a*,*l*]P also resulted in the formation of large amounts of DB[*a*,*l*]PDE−DNA adducts (Figure 3A,B, Table 2). In addition, considerable amounts of earlier-eluting highly polar DNA adducts were detected (Figure 3A,B, Table 2). Since human P450 1A1 is able to generate 8,9-, 11,12-, and 13,14-dihydrodiols during incubation of DB[*a*,*l*]P [(*5*) cf. above], it is possible that this P450 isoform catalyzes the formation of highly polar DB[*a*,*l*]P−DNA adducts via successive oxidations of different benzo rings in the peri-condensed hexacyclic molecule. Strong evidence in support of this conclusion is provided by a study recently presented by Nesnow et al. (*54*) that reported that human P450 1A1 is able to metabolically convert the 8,9-dihydrodiol of DB[*a*,*l*]P to a previously unknown tetrahydrotetrol. Furthermore, an unidentified tetrahydrotetrol epoxide−DNA adduct was detected after coincubation of human P450 1A1 with DB-[*a*,*l*]P-8,9-dihydrodiol in the presence of DNA (*54*). Involvement of multiple sites of metabolic activation of the hexacyclic DB[*a*,*l*]P could be anticipated based on similar findings during activation of certain other penta- or hexacyclic PAH such as B[*a*]P (*55*), dibenz[*a*,*h*]anthracene (DB[*a*,*h*]A) (*56*, *57*), and dibenzo[*a*,*h*]pyrene (*58*). These PAH have been shown to be capable of undergoing further oxidation after intermediate formation of vicinal dihydrodiol epoxides or to be electrophilically activated as tetrahydrotetrol epoxides via the formation of corresponding tetrahydrotetrol precursors.

Incubation of the immediate metabolic precursor of (−)-*anti*-DB[*a*,*l*]PDE, the (−)-(11*R*,12*R*)-dihydrodiol (Scheme 1), with V79 cells expressing human P450 1B1 indicated that this isoform metabolically generates (−)-*anti*-DB-

[2] Baird, W. M., Ralston, S. L., Coffing, S. L., Luch, A., and Seidel, A. Unpublished data.

[*a*,*l*]PDE−DNA adducts (Figure 5A) in extraordinary large amounts (Table 2). No other adducts have been detected. In contrast, V79 cells expressing human P450 1A1 are able to produce (−)-*anti*-DB[*a*,*l*]PDE as well as the highly polar DNA adducts (Figure 5B) previously detected after incubation of the parent compound. The similar HPLC patterns of highly polar DNA adducts obtained after incubation of DB[*a*,*l*]P or (−)-DB[*a*,*l*]P-11,-12-dihydrodiol with V79 cells stably expressing human P450 1A1 (cf. Figures 4A,B, 5B) provide strong evidence that the pathway(s) of successive oxidations at multiple sites of DB[*a*,*l*]P to form these polar DNA-binding metabolites include an epoxidation step at the 11,12-position.

The presence of highly polar DNA adducts together with DB[*a*,*l*]PDE−DNA adducts in V79 cells expressing human P450 1A1 (Figures 4A,B, 5B) and the absence of these polar DNA adducts in MCF-7 cells (*29*, *30*) and V79 cells expressing human P450 1B1 after exposure to DB-[*a*,*l*]P or its 11,12-dihydrodiol (Figures 3B, 5A) suggest that human P450 1A1 is not induced in MCF-7 cells by treatment with low doses of DB[*a*,*l*]P and that the DB-[*a*,*l*]PDE−DNA adducts found in MCF-7 cells result from activation of DB[*a*,*l*]P by human P450 1B1 which is constitutively expressed (*18*). Cai et al. (*59*) found that treatment of MCF-7 cells with DB[*a*,*l*]P increased the level of P450 1B1, but not the level of P450 1A1. The same result was obtained previously after incubation of MCF-7 cells or treatment of mouse skin with B[*c*]Ph (*60*). In contrast, treatment of MCF-7 cells with B[*a*]P induced both P450 1B1 and P450 1A1 (*59*). Typical inducers of P450 1A isoforms have been demonstrated to possess a large molecular area/depth ratio as a requirement for sufficient binding of the Ah receptor to cause induction of P450 1A enzymes (*61*, *62*). This requirement is fulfilled by compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), MC, or planar PAH like B[*a*]P or DB-[*a*,*h*]A (*61*, *62*). In contrast, nonplanar compounds with a small area/depth ratio have limited ability to induce P450 1A1; however, they have been shown to induce other P450 isoforms via an Ah receptor-independent pathway, e.g., members of the P450 2B subfamily which are preferentially involved in biotransformation of non-planar bulky compounds (*61*, *62*). Molecules containing a sterically hindered fjord region such as B[*c*]Ph and DB-[*a*,*l*]P exhibit an out-of plane distortion due to repulsive hydrogen interactions (*63*); thus, differences in the induction of P450 1A1 by B[*a*]P and DB[*a*,*l*]P might be due to their different ability to bind the Ah receptor. Further investigations are needed to clarify this point.

In conclusion, the present study demonstrates that the potent carcinogen DB[*a*,*l*]P is metabolically activated by human P450 1B1 very effectively via its (−)-(11*R*,12*R*)-dihydrodiol to the DNA-binding fjord region (−)-*anti*-DB-[*a*,*l*]PDE. Small amounts of (+)-*syn*-DB[*a*,*l*]PDE−DNA adducts are detectable only after exposure to high concentrations of the parent compound. Studies of V79 cells expressing human P450 1A1 have demonstrated that this isoform is also able to catalyze the formation of large amounts of DB[*a*,*l*]PDE−DNA adducts as well as highly polar DNA adducts which may result from oxidations at both the 8,9- and 11,12-positions of the parent PAH. However, taking into account that P450 1A1 is virtually inactive in human tissues in the absence of inducers (*64*, *65*), the similar DB[*a*,*l*]P−DNA adduct profiles obtained in V79 cells expressing human P450 1B1 and in human mammary carcinoma MCF-7 cells suggest that P450 1B1 may be responsible for activation of DB[*a*,*l*]P at low levels at which human exposure occurs.

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References

(1) Thakker, D. R., Levin, W., Yagi, H., Wood, A. W., Conney, A. H., and Jerina, D. M. (1988) Stereoselective biotransformation of polycyclic aromatic hydrocarbons to ultimate carcinogens. In *Stereochemical Aspects of Pharmacologically Active Compounds* (Wainer, A. W., and Dryer, D., Eds.) pp 271−296, Marcel Dekker, New York.

(2) Jacob, J., Raab, G., Soballa, V., Schmalix, W. A., Grimmer, G., Greim, H., Doehmer, J., and Seidel, A. (1996) Cytochrome P450-mediated activation of phenanthrene in genetically engineered V79 Chinese hamster cells. *Environ. Toxicol. Pharmacol.* **1**, 1−11.

(3) Shimada, T., Martin, M. V., Pruess-Schwartz, D., Marnett, L., and Guengerich, F. P. (1989) Roles of individual human cytochrome P-450 enzymes in the bioactivation of benzo[*a*]pyrene, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. *Cancer Res.* **49**, 6304−6312.

(4) Shou, M., Korzekwa, K. R., Crespi, C. L., Gonzalez, F. J., and Gelboin, H. V. (1994) The role of 12 cDNA-expressed human, rodent, and rabbit cytochromes P450 in the metabolism of benzo-[*a*]pyrene and benzo[*a*]pyrene *trans*-7,8-dihydrodiol. *Mol. Carcinogen.* **10**, 159−168.

(5) Shou, M., Krausz, K. W., Gonzalez, F. J., and Gelboin, H. V. (1996) Metabolic activation of the potent carcinogen dibenzo[*a*,*l*]pyrene by human recombinant cytochromes P450, lung and liver microsomes. *Carcinogenesis (London)* **17**, 2429−2433.

(6) Shou, M., Krausz, K. W., Gonzalez, F. J., and Gelboin, H. V. (1996) Metabolic activation of the potent carcinogen dibenz[*a*,*h*]anthracene by cDNA-expressed human cytochromes P450. *Arch. Biochem. Biophys.* **328**, 201−207.

(7) Doehmer, J., and Greim, H. (1993) Cytochromes P450 in genetically engineered cell cultures: the gene technological approach. In *Cytochrome P450* (Schenkman, J. B., and Greim, H., Eds.) pp 415−425, Springer-Verlag, Berlin.

(8) Doehmer, J. (1993) V79 Chinese hamster cells genetically engineered for cytochrome P450 and their use in mutagenicity and metabolism studies. *Toxicology* **82**, 105−118.

(9) Shen, Z., Liu, J., Wells, R. L., and Elkind, M. M. (1994) cDNA cloning, sequence analysis, and induction by aryl hydrocarbons of a murine cytochrome P450 gene, Cyp1b1. *DNA Cell Biol.* **13**, 763−769.

(10) Savas, U., Bhattacharyya, K. K., Christou, M., Alexander, D. L., and Jefcoate, C. R. (1994) Mouse cytochrome P450EF, representative of a new 1B subfamily of cytochrome P-450s: cloning, sequence determination, and tissue expression. *J. Biol. Chem.* **269**, 14905−14911.

(11) Pottenger, L. H., and Jefcoate, C. R. (1990) Characterization of a novel cytochrome P450 from the transformable cell line, C3H/10T1/2. *Carcinogenesis (London)* **11**, 321−327.

(12) Bhattacharyya, K. K., Brake, P. B., Eltom, S. E., Otto, S. A., and Jefcoate, C. R. (1995) Identification of a rat adrenal cytochrome P450 active in polycyclic aromatic hydrocarbon metabolism as rat CYP1B1: demonstration of a unique tissue specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. *J. Biol. Chem.* **270**, 11595−11602.

(13) Walker, N., Gastel, J. A., Costa, L. T., Clark, G. C., Lucier, G. W., and Sutter, T. (1995) Rat CYP1B1: an adrenal cytochrome P450 that exhibits sex-dependent expression in livers and kidneys of TCDD-treated animals. *Carcinogenesis (London)* **16**, 1319−1327.

(14) Sutter, T. R., Tang, Y. M., Hayes, C. L., Wo, Y. Y. P., Jabs, E. W., Li, X., Yin, H., Cody, C. W., and Greenlee, W. F. (1994) Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *J. Biol. Chem.* **269**, 13092−13099.

(15) Tang, Y. M., Wo, Y.-Y. P., Stewart, J., Hawkins, A. L., Griffin, C. A., Sutter, T. R., and Greenlee, W. F. (1996) Isolation and characterization of the human cytochrome P450 CYP1B1 gene. *J. Biol. Chem.* **271**, 28324−28330.

(16) Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., and Guengerich, F. P. (1996) Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res.* **56**, 2979−2984.

(17) Christou, M., Savas, U., Schroeder, S., Shen, X., Thompson, T., Gould, M. N., and Jefcoate, C. R. (1995) Cytochromes CYP1A1 and CYP1B1 in the rat mammary gland: Cell-specific expression and regulation by polycyclic aromatic hydrocarbons and hormones. *Mol. Cell. Endocrinol.* **115**, 41−50.

(18) Christou, M., Savas, Ü., Spink, D. C., Gierthy, J. F., and Jecoate, C. R. (1994) Coexpression of human CYP1A1 and human analogue of cytochrome P450-EF in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the human mammary carcinoma-derived MCF-7 cells. *Carcinogenesis (London)* **15**, 725−732.

(19) Otto, S., Bhattacharyya, K. K., and Jefcoate, C. R. (1995) Polycyclic aromatic hydrocarbon metabolism in rat adrenal, ovary, and testis microsomes is catalyzed by the same novel cytochrome P450 (P450RAP). *Endocrinology* **131**, 3067−3076.

(20) Seidel, A., Soballa, V. J., Raab, G., Frank, H., Greim, H., Grimmer, G., Jacob, J., and Doehmer, J. (1997) Regio- and stereoselectivity in the metabolism of benzo[*c*]phenanthrene mediated by genetically engineered V79 Chinese hamster cells expressing rat and human cytochromes P450. *Environ. Toxicol. Pharmacol.*, in press.

(21) Snook, M. E., Severson, R. F., Arrendale, R. F., Higman, H. C., and Chortyk, O. T. (1977) The identification of high molecular weight polynuclear aromatic hydrocarbons in a biologically active fraction of cigarette smoke condensate. *Beitr. Tabakforsch.* **9**, 79−101.

(22) Kozin, I. S., Gooijer, C., and Velthorst, N. H. (1995) Direct determination of dibenzo[*a*,*l*]pyrene in crude extracts of environmental samples by laser excited Shpol'skii spectroscopy. *Anal. Chem.* **67**, 1623−1626.

(23) DeRatt, W. K., Kooijman, S. A. L. M., and Gielen, J. W. J. (1987) Concentrations of polycyclic hydrocarbons in airborne particles in Netherlands and their correlation with mutagenicity. *Sci. Total. Environ.* **66**, 95−114.

(24) Mumford, J. L., Harris, D. B., Williams, K., Chuang, J. C., and Cooke, M. (1987) Indoor air sampling and mutagenicity studies of emissions from unvented coal combustion. *Environ. Sci. Technol.* **21**, 308−311.

(25) Mumford, J. L., Xueming, L., Fuding, H., Xu, B. L., and Chuang, J. C. (1995) Human exposure and dosimetry of polycyclic aromatic hydrocarbons in urine from Xuan Wei, China with high lung cancer mortality associated with exposure to unvented coal smoke. *Carcinogenesis (London)* **16**, 3031−3036.

(26) Cavalieri, E. L., Higginbotham, S., RamaKrishna, N. V. S., Devanesan, P. D., Todorovic, R., Rogan, E. G., and Salmasi, S. (1991) Comparative dose−response tumorigenicity studies of dibenzo[*a*,*l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene, benzo[*a*]pyrene and two dibenzo[*a*,*l*]pyrene dihydrodiols in mouse skin and rat mammary gland. *Carcinogenesis (London)* **12**, 1939−1944.

(27) Higginbotham, S., RamaKrishna, N. V. S., Johansson, S. L., Rogan, E. G., and Cavalieri, E. L. (1993) Tumor-initiating activity and carcinogenicity of dibenzo[*a*,*l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene at low doses in mouse skin. *Carcinogenesis (London)* **14**, 875−878.

(28) LaVoie, E. J., He, Z.-M., Meegalla, R. L., and Weyand, E. H. (1993) Exceptional tumor-initiating activity of 4-fluorobenzo[*j*]fluoranthene on mouse skin: comparison with benzo[*j*]fluoranthene, 10-fluorobenzo[*j*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene and 7,12-dimethylbenz[*a*]anthracene. *Cancer Lett.* **70**, 7−14.

(29) Ralston, S. L., Lau, H. H. S., Seidel, A., Luch, A., Platt, K. L., and Baird, W. M. (1994) The potent carcinogen dibenzo[*a*,*l*]pyrene is metabolically activated to fjord-region 11,12-diol 13,14-epoxides in human mammary carcinoma MCF-7 cell cultures. *Cancer Res.* **54**, 887−890.

(30) Ralston, S. L., Seidel, A., Luch, A., Platt, K. L., and Baird, W. M. (1995) Stereoselective activation of dibenzo[*a*,*l*]pyrene to (−)-anti-(11*R*,12*S*,13*S*,14*R*)- and (+)-syn(11*S*,12*R*,13*S*,14*R*)-11,12-diol-13,-14-epoxides which bind extensively to deoxyadenosine residues of DNA in the human mammary carcinoma cell line MCF-7. *Carcinogenesis (London)* **16**, 2899−2907.

(31) Li, K.-M., Todorovic, R., Rogan, E. G., Cavalieri, E. L., Ariese, F., Suh, M., Jankowiak, R., and Small, G. J. (1995) Identification and quantitation of dibenzo[*a*,*l*]pyrene-DNA adducts formed by rat liver microsomes in vitro: preponderance of depurination adducts. *Biochemistry* **34**, 8043−8049.

(32) Luch, A., Seidel, A., Glatt, H.-R., and Platt, K. L. (1997) Metabolic activation of the (+)-*S*,*S*- and (−)-*R*,*R*-enantiomers of *trans*-11,12-dihydroxy-11,12-dihydrodibenzo[*a*,*l*]pyrene: stereoselectivity, DNA adduct formation, and mutagenicity in Chinese hamster V79 cells. *Chem. Res. Toxicol.* **10**, 1161−1170.

(33) Arif, J. M., and Gupta, R. C. (1997) Microsome-mediated bioactivation of dibenzo[*a*,*l*]pyrene and identification of DNA adducts by ^{32}P-postlabeling. *Carcinogenesis (London)* **18**, 1999−2007.

(34) Luch, A., Glatt, H.-R., Platt, K. L., Oesch, F., and Seidel, A. (1994) Synthesis and mutagenicity of the diastereomeric fjord-region 11,-12-dihydrodiol 13,14-epoxides of dibenzo[*a*,*l*]pyrene. *Carcinogenesis (London)* **15**, 2507−2516.

(35) Amin, S., Desai, D., Dai, W., Harvey, R. G., and Hecht, S. S. (1995) Tumorigenicity in newborn mice of fjord region and other sterically hindered diol epoxides of benzo[*g*]chrysene, dibenzo[*a*,*l*]-pyrene (dibenzo[*def*,*p*]chrysene), 4H-cyclopenta[*def*]chrysene and fluoranthene. *Carcinogenesis (London)* **16**, 2813−2817.

(36) Amin, S., Krzeminski, J., Rivenson, A., Kurtzke, C., Hecht, S. S., and El-Bayoumy, K. (1995) Mammary carcinogenicity in female CD rats of fjord region diol epoxides of benzo[*c*]phenanthrene, benzo[*g*]chrysene and dibenzo[*a*,*l*]pyrene. *Carcinogenesis (London)* **16**, 1971−1974.

(37) Gill, H. S., Kole, P. L., Wiley, J. C., Li, K. M., Higginbotham, S., Rogan, E. G., and Cavalieri, E. L. (1994) Synthesis and tumor-initiating activity in mouse skin of dibenzo[*a*,*l*]pyrene *syn*- and *anti*-fjord-region diolepoxides. *Carcinogenesis (London)* **15**, 2455−2460.

(38) Ralston, S. L., Coffing, S. L., Seidel, A., Luch, A., Platt, K. L., and Baird, W. M. (1997) Stereoselective activation of dibenzo-[*a*,*l*]pyrene and its *trans*-11,12-dihydrodiol to fjord region 11,12-diol 13,14-epoxides in a human mammary carcinoma MCF-7 cell-mediated V79 cell mutation assay. *Chem. Res. Toxicol.* **10**, 687−693.

(39) Frank, H., Luch, A., Oesch, F., and Seidel, A. (1996) 4-[4-(Dimethylamino)-phenylazo]benzoate, a new red-shifted chromophore for use in the exciton chirality method: assignment of absolute configuration to fjord-region metabolites of dibenzo[*a*,*l*]-pyrene. *Polycycl. Aromat. Compd.* **10**, 109−116.

(40) Glatt, H.-R., Gemperlein, I., Turchi, G., Heinritz, H., Doehmer, J., and Oesch, F. (1987) Search for cell culture systems with diverse xenobiotic-metabolizing activities and their use in toxicological studies. *Mol. Toxicol.* **1**, 313−334.

(41) Doehmer, J., Dogra, S., Friedberg, T., Monier, S., Adesnik, M., Glatt, H.-R., and Oesch, F. (1988) Stable expression of rat cytochrome P-450IIB1 cDNA in Chinese hamster cells (V79) and metabolic activation of aflatoxin B_1. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5769−5773.

(42) Schneider, A., Schmalix, W. A., Siruguri, V., de Groene, E. M., Horbach, G. J., Kleingeist, B., Lang, D., Boecker, R., Belloc, C., Beaune, P., Greim, H., and Doehmer, J. (1996) Stable expression of human cytochrome P450 3A4 in conjunction with human NADPH-cytochrom P450 oxidoreductase in V79 Chinese hamster cells. *Arch. Biochem. Biophys.* **332**, 295−304.

(43) Graham, F. L., and Van der Eb, A. J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456−467.

(44) Schmalix, W. A., Lang, D., Schneider, A., Bocker, R., Greim, H., and Doehmer, J. (1996) Stable expression and coexpression of human cytochrome P450 oxidoreductase and cytochrome P450 1A2 in V79 Chinese hamster cells: sensitivity to quinons and biotransformation of 7-alkoxyresorufins and triazines. *Drug Metab. Dispos.* **24**, 1314−1319.

(45) Lau, H. H. S., and Baird, W. M. (1991) Detection and identification of benzo[*a*]pyrene-DNA adducts by [^{35}S]phosphorothioate labeling and HPLC. *Carcinogenesis (London)* **12**, 885−893.

(46) Devanesan, P. D., Cremonesi, P., Nunnally, J. E., Rogan, E. G., and Cavalieri, E. L. (1990) Metabolism and mutagenicity of dibenzo[*a*,*e*]pyrene and the very potent environmental carcinogen dibenzo[*a*,*l*]pyrene. *Chem. Res. Toxicol.* **3**, 580−586.

(47) Parkinson, A., Safe, S. H., Robertson, L. W., Thomas, P. E., Ryan, D. E., Reik, L. M., and Levin, W. (1983) Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. *J. Biol. Chem.* **258**, 5967−5976.

(48) Thomas, P. E., Reik, L. M., Ryan, D. E., and Levin, W. (1983) Induction of two immunochemically related rat liver cytochrome P-450 isozymes, cytochromes P-450c and P-450d, by structurally diverse xenobiotics. *J. Biol. Chem.* **258**, 4590−4598.

(49) Nesnow, S., Davis, C., Nelson, G., Ross, J. A., Allison, J., Adams, L., and King, L. C. (1997) Comparison of the morphological transforming activities of dibenzo[*a*,*l*]pyrene and benzo[*a*]pyrene in C3H10T1/2CL8 cells and characterization of the dibenzo[*a*,*l*]-pyrene-DNA adducts. *Carcinogenesis (London)* **18**, 1973−1978.

(50) Prahalad, A. K., Ross, J. A., Nelson, G. B., Roop, B. C., King, L. C., Nesnow, S., and Mass, M. J. (1997) Dibenzo[*a*,*l*]pyrene-induced DNA adduction, tumorigenicity, and Ki-*ras* oncogene mutations in strain A/J mouse lung. *Carcinogenesis (London)* **18**, 1955−1963.

(51) Crespi, C. L., Penman, B. W., Steimel, D. T., Smith, T., Yang, C. S., and Sutter, T. R. (1997) Development of a human lymphoblastoid cell line constitutively expressing human CYP1B1 cDNA: substrate specificity with model substrates and promutagens. *Mutagenesis* **12**, 83−89.

(52) Sayer, J. M., Yagi, H., Silverton, J. V., Friedman, S. L., Whalen, D. L., and Jerina, D. M. (1982) Conformational effects in the hydrolyses of rigid benzylic epoxides: implications for diol epoxides of polycyclic hydrocarbons. *J. Am. Chem. Soc.* **104**, 1972−1978.

(53) Sayer, J. M., Whalen, D. L., Friedman, S. L., Paik, A., Yagi, H., Vyas, K. P., and Jerina, D. M. (1984) Conformational effects in the hydrolysis of benzo-ring diol epoxides that have bay-region diol groups. *J. Am. Chem. Soc.* **106**, 226−233.

(54) Nesnow, S., Davis, C., Padgett, W., George, M., Lambert, G., Adams, L., and King, L. (1997) Biotransformation and DNA adduct formation of 8,9-dihydroxy-8,9-dihydrodibenzo[*a,l*]pyrene by induced rat liver and human CYP1A1 and CYP1B1 microsomes. 16th International Symposium on Polycyclic Aromatic Compounds, Nov 4−8, Charlotte, NC.

(55) Dock, L., Waern, F., Martinez, M., Grover, P. L., and Jernstrom, B. (1986) Studies on the further activation of benzo[*a*]pyrene diol epoxides by rat liver microsomes and nuclei. *Chem.-Biol. Interact.* **58**, 301−318.

(56) Carmichael, P. L., Platt, K. L., Shé, M. N., Lecoq, S., Oesch, F., Phillips, D. H., and Grover, P. L. (1993) Evidence for the involvement of a bis-diol-epoxide in the metabolic activation of dibenz[*a,h*]anthracene. *Cancer Res.* **53**, 944−948.

(57) Fuchs, J., Mlcoch, J., Platt, K. L., and Oesch, F. (1993) Characterization of highly polar bis-dihydrodiol epoxide-DNA adducts formed after metabolic activation of dibenz[*a,h*]anthracene. *Carcinogenesis (London)* **14**, 863−867.

(58) Marsch, G. A., Jankowiak, R., Small, G. J., Hughes, N. C., and Phillips, D. H. (1992) Evidence of involvement of multiple sites of metabolism in the in vivo covalent binding of dibenzo[*a,h*]-pyrene to DNA. *Chem. Res. Toxicol.* **5**, 765−772.

(59) Cai, Y., Marcus, C., Guengerich, F. P., Gelboin, H. V., and Baird, W. M. (1997) Roles of cytochrome P450 1A1 and 1B1 in metabolic activation of dibenzo[*a,l*]pyrene by microsomes from the human mammary carcinoma cell line MCF-7. 16th International Symposium on Polycyclic Aromatic Compounds, Nov 4−8, Charlotte, NC.

(60) Einolf, H. J., Story, W. T., Marcus, C. B., Larsen, M. C., Jefcoate, C. R., Greenlee, W. F., Yagi, H., Jerina, D. M., Amin, S., Park, S. S., Gelboin, H. V., and Baird, W. M. (1997) Role of cytochrome P450 enzyme induction in the metabolic activation of benzo[*c*]-phenanthrene in human cell lines and mouse epidermis. *Chem. Res. Toxicol.* **10**, 609−617.

(61) Ioannides, C., and Parke, D. V. (1993) Induction of cytochrome P4501 as an indicator of potential chemical carcinogenesis. *Drug Metab. Rev.* **25**, 485−501.

(62) Ioannides, C., and Parke, D. V. (1990) The cytochrome P450 I gene familiy of microsomal hemoproteins and their role in the metabolic activation of chemicals. *Drug Metab. Rev.* **22**, 1−85.

(63) Hirshfeld, F. L. (1963) The structure of overcrowded aromatic compounds. Part VII. Out-of plane deformation in benzo[*c*]-phenanthrene and 1,12-dimethylbenzo[*c*]phenanthrene. *J. Chem. Soc.* 2126−2135.

(64) Guengerich, F. P., and Shimada, T. (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.* **4**, 391−407.

(65) Gonzalez, F. J. (1993) Cytochromes P450 in humans. In *Cytochrome P450* (Schenkman, J. B., and Greim, H., Eds.) pp 239−257, Springer-Verlag, Berlin.

(66) Döhr, O., Vogel, C., and Abel, J. (1995) Different response of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-sensitive genes in human breast cancer MCF-7 and MDA-MB 231 cells. *Arch. Biochem. Biophys.* **321**, 405−412.

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