The stereoselectivity of the oxidation of 7,8-dihydrobenzo[a]pyrene (H2BP) to 9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (HBP-epoxide) by prostaglandin H synthase and cytochrome P-450 has been studied using microsomal preparations from ram seminal vesicles and rat liver. Incubations were performed in the presence of polyguanylic acid and the adducts formed between HBP-epoxide and guanosine were isolated following the recovery and hydrolysis of the poly(G). When (+)-HBP-epoxide was reacted with poly(G), four diastereomeric adducts were formed by the cis and trans addition of the exocyclic amino group of guanine to the benzylic carbon of the epoxide enantiomers. Each diastereomer was identified by a combination of ultraviolet, nuclear magnetic resonance, circular dichroism, and mass spectroscopy. Under comparable conditions, ram seminal vesicle microsomes in the presence of arachidonic acid triggered the binding of H2BP to poly(G) to a greater extent than rat liver microsomes from untreated and phenobarbital- and methylcholanthrene pretreated animals in the presence of NADPH. Quantitation of the (-)-cis- and (+)-cis-guanosine adducts revealed the degree of stereoselectivity of epoxidation. The ratio of (-)/(+): adducts was 64:36 for PGH synthase and 89:11 (control), 62:38 (phenobarbital), and 69:31 (methylcholanthrene) for cytochrome P-450-catalyzed reactions. PGH synthase catalyzed the epoxidation of H2BP with little or no stereoselectivity in contrast to cytochrome P-450. The utility of the poly(G) binding technique for the elucidation of the stereoselective generation of chiral electrophiles is discussed along with the mechanistic implications of the results.

Many environmental chemicals exert toxic effects as a result of metabolism (1-4). The polycyclic aromatic hydrocarbon, BP,1 is converted to highly mutagenic and carcinogenic derivatives following enzymatic oxidation (5-7). Several studies have shown that dihydrodiol epoxides are the most mutagenic and carcinogenic metabolites of BP (4, 8-11). These are formed by the epoxidation of the proximate carcinogen, BP-7,8-diol (Equation 1) (12, 13). Epoxidation can be catalyzed by mixed function oxidases (13-18), PGH synthase (19-23), and carcinogenic metabolites of BP (Equation 2). Since the starting material cannot be resolved, the stereoselectivity and carcinogenic potency (29-36). In the case of BP-7,8-diol, the stereochecmistry of epoxidation has been elucidated by metabolizing optically pure enantiomers resolved from (+)-BP-7,8-diol and analyzing the enantiomeric epoxide hydrolysis products formed (15-17, 37). We have developed an alternative method for determining the stereochecmistry of epoxidation which utilizes a chiral nucleophile, poly(G), to trap enantiomeric epoxides (38). After hydrolysis to nucleosides, the resulting diastereomeric adducts are separated and quantitated by HPLC (39). Using this technique, we have shown that PGH synthase oxidizes both enantiomers of (+)-BP-7,8-diol to an equal mixture of enantiomers of diol epoxide 2 (38).

H2BP is an achiral molecule, but can be converted to enantiomeric H2BP-epoxides and, therefore, is enantiotopic (Equation 2). Since the starting material cannot be resolved, the reactivity toward nucleic acids and in their mutagenic and carcinogenic potency (29-36). In the case of BP-7,8-diol, the stereochemistry of epoxidation has been elucidated by metabolizing optically pure enantiomers resolved from (+)-BP-7,8-diol and analyzing the enantiomeric epoxide hydrolysis products formed (15-17, 37). We have developed an alternative method for determining the stereochemistry of epoxidation which utilizes a chiral nucleophile, poly(G), to trap enantiomeric epoxides (38). After hydrolysis to nucleosides, the resulting diastereomeric adducts are separated and quantitated by HPLC (39). Using this technique, we have shown that PGH synthase oxidizes both enantiomers of (+)-BP-7,8-diol to an equal mixture of enantiomers of diol epoxide 2 (38).

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our chiral nucleophile approach is uniquely suited to define the stereochemistry of the intermediate epoxides.

The present study was undertaken in order to compare the stereochemistry of epoxidation of H\(_2\)BP by PGH synthase and by the mixed function oxidase RLM. As a result of this study, we are now assigning not only the relative stereochemistry of oxidation by both enzymes but also the absolute stereochemistry of the nucleoside adducts.

**EXPERIMENTAL PROCEDURES**

**Materials**—H\(_2\)BP and H\(_4\)BP-epoxide were synthesized by a modification of published procedures (26, 40). H\(_2\)BP-7-one was purchased from Aldrich. [\(\text{H}^3\)]BP-7,8-diol was obtained from the Chemical Repository of the National Cancer Institute. Poly(G), alkaline phosphatase, and NADPH were purchased from Sigma. 204 was from NuChek Prep, Elysian, MN. HPLC grade solvents were purchased from Fisher. All other chemicals were of reagent grade and were from Fisher.

**Enzyme Preparations**—RSVM were prepared as described previously (41). RLM were prepared by the procedure of Tuneke et al. (42). Microsomes from induced animals were prepared from animals injected intraperitoneally with MC (25 mg/kg) in corn oil or PB diluting with 200 ml of water, the reaction mixture was extracted with ethyl acetate with a retention time of 31 min. The two compounds exhibited loss of aniline, 271 and 252 (25%) were found. The NMR spectra of the two compounds were recorded as stated above.

**Acid Hydrolysis of H\(_2\)BP-epoxide-Guanosine Adducts**—The diastereomeric adducts (100 nmol) were individually hydrolyzed in 0.05 M HCl by heating at 37°C for 18 h. The solutions were neutralized with aqueous sodium bicarbonate solution and extracted with ethyl acetate. The ethyl acetate extract was dried over anhydrous magnesium sulfate and the solvent was evaporated. Analysis of the residue by HPLC on an Ultrasphere ODS column under conditions described for \(\text{H}_2\)BP-epoxide-guanosine adduct gave two peaks with retention times of 37 and 51 min.

**Attempted Base Hydrolysis of H\(_2\)BP-Guanosine Adducts**—A mixture of purified H\(_2\)BP-epoxide-guanosine adduct (containing ~100 nmol of each adduct) was heated in 1 ml of 1 M KOH at 100°C for 1 h. Duplicate incubations were combined for LH-20 separation and analysis. The adducts were separated into four peaks with retention times of 31, 36, 38, and 44 min. The retention times were approximately the same on the analytical or preparative column.

**Acid Hydrolysis of H\(_2\)BP-epoxide-Guanosine Adducts**—The diastereomeric adducts (~100 nmol) were individually hydrolyzed in 0.05 M HCl by heating at 37°C for 18 h. The solutions were neutralized with aqueous sodium bicarbonate solution and extracted with ethyl acetate. The ethyl acetate extract was dried over anhydrous magnesium sulfate and the solvent was evaporated. Analysis of the residue by HPLC on an Ultrasphere ODS column under conditions described for \(\text{H}_2\)BP-epoxide-guanosine adduct gave two peaks with retention times of 37 and 51 min.

**Determination of k\(_\text{pK}_a\) Values of H\(_2\)BP-epoxide-Guanosine Adducts**—The \(k\text{pK}_a\) values of the \(\text{H}_2\)BP-epoxide adducts were determined by the method of Moore and Koreeda (45). The adducts (~0.3 A) were partitioned between 1 ml buffer solutions of pH 1.0-12.0 and 1 ml of 25% 1-butanol in ethyl acetate. The amounts of adduct in the aqueous phase were measured spectrophotometrically (45).

**NMR Spectra**—NMR spectra of H\(_2\)BP-epoxide-guanosine adducts and 9-acetoxy-10-anilino-7,8,9,10-tetrahydrobenzo[a]pyrene were recorded from the method of Moore and Koreeda (45). The adducts (~0.3 A) were partitioned between 1 ml buffer solutions of pH 1.0-12.0 and 1 ml of 25% 1-butanol in ethyl acetate. The amounts of adduct in the aqueous phase were measured spectrophotometrically (45).

**CD Spectra**—CD spectra of H\(_2\)BP-epoxide-guanosine adducts were taken in methanol on a Jasco-40 spectropolarimeter equipped with a Nova-3 data processor and a Tracor Northern digital signal processor.

**Mass Spectra**—The field desorption mass spectrum of an underviolated, H\(_2\)BP-epoxide-guanosine adduct (peak B) was recorded on a Varian Mat CH-5 double-focusing mass spectrometer.

**Metabolism of H\(_2\)BP and Poly(G) Binding in the Presence of RSVM**—Incubation mixtures contained 500 nmol of H\(_2\)BP, 5 mg of poly(G), ~5 mg of RSVM protein, and 20 ml of acetone in a total volume of 5 ml of 0.01 M Na\(_2\)HPO\(_4\) (pH 7.5). The reaction was initiated by adding the enzyme. The reaction was stopped by the addition of 5 ml of phenol reagent (phenol (500 g), m-cresol (70 ml), water (5 ml), and 8-hydroxyquinoline (0.5 g)). Two ml of 0.10 M NaCl was added and the samples were extracted as before (38). The aqueous layers were combined and extracted with ethyl acetate (3 x 5 ml). The poly(G) was reisolated and digested to guanosine adducts according to the procedure of Moore et al. (44). The adducts were purified by Sephadex LH-20 chromatography and analyzed by HPLC under conditions described for the separation of H\(_2\)BP-epoxide-guanosine adducts. Duplicate incubations were combined for LH-20 separation and HPLC analysis. The individual peaks were collected and UV-Vis spectra were recorded. The adducts were quantitated from their absorbance at 434 nm using an extinction coefficient of 37,000 for the tetrahydrobenzo[a]pyrene moiety (44). The peaks were separately cochromatographed with standards to establish their identity.

**Metabolism of H\(_2\)BP and Poly(G) Binding in the Presence of Rat Liver Microsomes**—Incubation mixtures contained 500 nmol of H\(_2\)BP, 5 mg of poly(G), 5-6 mg of microsomal protein (MC-induced, PB-induced, or control), and 20 ml of acetone in a total volume of 5 ml of 0.01 M Na\(_2\)HPO\(_4\) (pH 7.5), containing 10 mM MgCl\(_2\) and 10 mM MnCl\(_2\). The reaction was initiated by the addition of NADPH (0.5 mm). The incubations were carried out for 30 min at 37°C. The reactions were stopped by the addition of phenol reagent and worked up as indicated. The reisolated poly(G) was hydroxylated and adducts were quantitated as described above.

**Metabolism of BP-7,8-diol and Poly(G) Binding in the Presence of MC-induced Rat Liver Microsomes**—BP-7,8-diol metabolism was measured by guest on March 25, 2020http://www.jbc.org/Downloaded from
H$_2$BP Epoxidation by PGH Synthase and Cytochrome P-450

RESULTS

H$_2$BP-epoxide was found to bind to poly(G) at neutral pH. Binding was characterized by the UV absorption pattern of the 7,8,9,10-tetrahydrobenzo[a]pyrene moiety in the 320-355 nm region and was similar to that observed for the binding of poly(G) to diolepoxides (38). Digestion of the hydrocarbon-bound poly(G) to nucleosides followed by HPLC separation on an Ultrasphere ODS column gave four peaks which are shown in Fig. 1. The peaks do not co-chromatograph with the diole hydrolysis products of H$_2$BP-epoxide. The HPLC profile in Fig. 1 shows that the products formed consist of two groups based on their peak area and suggests that the peaks of equal intensity are formed by trans or cis opening of the enantio- mers of H$_2$BP-epoxide. The structures of the four peaks in Fig. 1 were deduced by spectral and chemical methods.

Samples of the four peaks were collected and their UV-Vis spectra were recorded in methanol. The peaks gave identical spectra which were similar to those of H$_2$BP-diols and are characteristic of the presence of a tetrahydrobenzo[a]pyrene chromophore. Analysis of the peaks by low resolution field desorption mass spectrometry provided information on their molecular weight. Fig. 2 is the field desorption mass spectrum of peak B which shows prominent ions at m/e 576 (84%), 558 (14%), and 444 (100%).

The ion at 576 represents a H$_2$BP-epoxide-guanosine adduct plus sodium. The fragment ions at 558 and 444 correspond to the loss of water and the loss of ribose with the transfer of a proton, respectively. The latter provides additional evidence for a guanosine derivative. Another ion seen at m/e 426 (16%) is consistent with the loss of a guanine moiety from the molecule. Other minor ions at m/e 306 (2%) and m/z 370 (2%) correspond to guanosine + Na and loss of guanosine and a proton from the molecule, respectively.

The CD spectra of the four peaks are shown in Fig. 3. The spectra of pairs of peaks A and B and C and D are essentially mirror images. On this basis, it can be reasonably concluded that the four peaks A to D are diastereomers. The spectra are very similar to those of diolepoxide-guanosine adducts (39). This gives further evidence that peaks A to D are formed by the reaction of guanosine with H$_2$BP-epoxide. The pairs of diastereomers with mirror image CD spectra would result from the cis or trans opening of the oxirane ring at C-10 of H$_2$BP-epoxide. Thus, the pairs AB and CD should represent either the cis or trans pair of possible guanosine adducts formed from H$_2$BP-epoxide.

The cis/trans stereochemistry of the four adducts was assigned by NMR spectroscopy. Fig. 4 displays the NMR spectra of adducts A and D in the region 85.5-9.0 ppm. The pairs of diastereomers gave virtually identical NMR spectra in this region. The major differences in the NMR spectra of A and D are due to differences in the chemical shift value for the C-10 proton. The C-10 proton signal in the NMR spectrum of A (85.95 ppm) is shifted downfield in the spectrum of D (86.41 ppm). Table I gives a comparison of chemical shift values for the C-10 proton of guanosine adducts, aniline adducts, and cis- and trans-H$_2$BP-diols. In the case of H$_2$BP-diols, the C-10 proton of the cis-isomer is shifted downfield compared to the trans and this has been confirmed by authentic synthesis of cis- and trans-H$_2$BP diols (39). The downfield shift for the C-10 proton in the cis isomer is in agreement with previous studies on NMR spectra of guanosine, adenosine, methanol, phenol, and aniline adducts derived from diolepoxides (43, 46, 47).

The position of substitution of the guanosine moiety in compounds A to D is deduced from the NMR spectra and by chemical methods. The NMR spectra of A and D in acetone (Fig. 4) show a doublet for the N$^2$ proton at 6.48 and 6.54 ppm, respectively, due to coupling to the C-10 proton. The N$^2$ proton signal and its coupling to the C-10 proton, is eliminated by deuterium exchange in CD$_3$OD. The possibility of substitution at C-8 of guanine was excluded by the presence of the C-8 proton signal at 7.9 ppm.

In order to provide further evidence for the N$^2$ substitution, compounds A to D were treated with 0.05 M HCl. They were completely hydrolyzed in 30 min. This is in agreement with the data of Moore et al. (44) on the acid hydrolysis of diolepoxide-guanosine adducts. The products of hydrolysis were identified as cis- and trans-H$_2$BP-diols and a small amount of H$_2$BP-9-one. This suggests that the site of attachment of guanosine to the H$_2$BP moiety is through an oxygen or nitro-
Fig. 3. CD spectra of H₃BP-epoxide-guanosine adducts. a, CD spectra of adducts A and B; b, CD spectra of adducts C and D. The spectra were recorded in methanol. ΔΕ is based on an extinction coefficient of 37,000 at 344 nm.

Fig. 4. NMR spectra of H₃BP-epoxide-guanosine adducts in the region 7.5—9.0 ppm. a, NMR spectrum of adduct A; b, NMR spectrum of adduct D. The spectra were recorded in acetone-d₆.

**Table 1**

| Compound                        | C-10 proton (δ ppm) |
|---------------------------------|---------------------|
| cis-9,10-Diol                   | 5.57                |
| trans-9,10-Diol                 | 5.45                |
| 9-Acetoxy-10-anilino-BP-1(cis)  | 5.89                |
| 9-Acetoxy-10-anilino-BP-1(trans) | 5.57               |
| Adduct A ((−)-trans)            | 5.95                |
| Adduct B ((+)-trans)            | 5.89                |
| Adduct C ((−)-cis)              | 6.43                |
| Adduct D ((+)-cis)              | 6.41                |

* cis-9,10-Dihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene.
† trans-9,10-Dihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene.
‡ 9-Acetoxy-10-anilino-7,8,9,10-tetrahydrobenzo[a]pyrene. The assignment in the parentheses is based on chemical shift values for C-10 proton.

The stereochemistry of epoxidation of H₂BP to H₃BP-epoxide was studied using poly(G) binding experiments. In order to validate the use of this method in determining sterechemistry of epoxidation, we have analyzed poly(G) binding derivatives from (±)-BP-7,8-diol in the presence of MC-induced RLM preparations and NADPH. It has been shown that the (+) and (−)-enantiomers of BP-7,8-diol are oxidized to (+)-diolepoxide 1 and (+)-diolepoxide 2 in the presence of a purified and reconstituted monoxygenase system (16). Further, the absolute stereochemistry of the guanosine adducts resulting from individual enantiomers of diolepoxide 1 and diolepoxide 2 has been determined (44). Fig. 5A is the HPLC profile of guanosine adducts resulting from the reaction of (±)-diolepoxide 1 and (±)-diolepoxide 2 with poly(G). Fig. 5B is the HPLC profile obtained when poly(G) was added to incubation mixtures containing (±)-[³H]BP-7,8-diol, MC-induced RLM, and NADPH. The radioactive peaks in Fig. 5B

*Gen to the benzylic carbon because a carbon-carbon bond would not be expected to be acid-labile. When compounds A to D were heated in 1.0 M KOH at 100 °C for 1 h, only minor degradation was observed. N¹-, N³-, N⁷-, and O⁶-substituted guanosines are degraded on base treatment while N²-substituted guanosine is stable toward base (44, 48, 49). Determination of the pKₐ values of adduct B by partition analysis established the presence of a free amidic proton at N¹ (45). Adduct B exhibited a basic pKₐ at pH ~ 1.5 and an acidic pKₐ at pH ~ 9.5. The above results, in combination with the NMR spectra of the adducts eliminates N¹, N³, N⁷, O⁶, and C⁸ as possible sites of substitution and suggests that the site of attachment of guanosine to the H₄BP moiety is through the N² amino group.

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co-chromatograph with four guanosine adducts derived from diolepoxides. Table II illustrates that they are derived from the (+)-enantiomer of diolepoxy 1 and the (+)-enantiomer of diolepoxy 2 and that they are formed in almost a 1:1 ratio. Levin et al. (16) have shown that in the presence of the purified MC-inducible P-450 monooxygenase system (±)-BP-7,8-diol is oxidized to give 42% diolepoxy 1-derived products and 58% diolepoxy 2-derived products. They have also shown that (+)-BP-diol gives predominantly diolepoxy 1 (97.5%) and the (−)-BP-7,8-diol gives diolepoxy 2 (82%).

Deusch et al. (15, 37) and Belvedere et al. (50) have shown that (−)-BP-7,8-diol is oxidized to diolepoxy 2 by certain forms of PB- or β-naphthoflavone-inducible purified rabbit liver microsomes. These results are in good agreement with the data obtained from our poly(G) binding studies in the presence of MC-RLM and NADPH. The peaks in Fig. 5B with retention times at 30 min and 45 min are (−)-cis and (+)-trans adducts, respectively, from (+)-diolepoxy 2 and peaks with retention times at 43 min and 36 min are (+)-cis and (−)-trans adducts, respectively, from (+)-diolepoxy 1. This demonstrates that our poly(G) trapping method gives results which are comparable to those obtained using conventional methods.

Fig. 6a is the HPLC profile of the guanosine adducts formed when poly(G) was added to incubation mixtures containing H2BP, RSVM, and 20:4. The profile shows that the pairs of diastereomeric adducts are formed in almost equal amounts. In other words, both enantiomers of the epoxide are formed from the hydrocarbon. This is in agreement with our previous findings on the poly(G) binding to BP-7,8-diol in the presence of PGH synthase (38).

Fig. 6b represents the HPLC profile of the guanosine adducts resulting from incubation of poly(G) with H2BP in the presence of microsomal preparations from control rat liver and NADPH. It shows that the predominant guanosine adduct formed is the (−)-cis isomer (co-chromatographic with peak C in Fig. 1). The adduct profiles obtained from the reaction of H2BP and poly(G) in the presence of NADPH and PB-induced RLM are qualitatively similar to the profile in Fig. 6b. This clearly demonstrates that NADPH-dependent mixed function oxidases oxidize H2BP to a specific enantiomer of H2BP-epoxide. A comparison of the HPLC profiles in Fig. 6 graphically illustrates the differences in the stereochemistry of epoxidation by PGH synthase and cytochromes P-450.

The amounts of guanosine adducts obtained from H2BP and poly(G) were quantitated from their absorption spectra. In all cases, the cis- and trans-diastereomers are formed in a ratio of 3:1. In some cases the trans adducts are formed in very small amounts and in such cases a combination of peak height and absorbance at 344 nm were used for quantitation. Since (−)-cis- and (+)-cis-diastereomers are produced from the two enantiomers of the epoxide, a comparison of the percentage of the cis-isomer is sufficient to demonstrate the stereochemistry of epoxidation. Table III is a comparison of the percentages of cis-isomers formed in the presence of PGH synthase and cytochromes P-450. The results indicate that the mixed function oxidases epoxidize H2BP stereoselectively while PGH synthase-dependent epoxidation is nonstereoselective.

A comparison of the relative amount of adducts formed in the presence of PGH synthase and cytochromes P-450 under identical incubation conditions. H2BP concentration, and microsomal protein concentration is also presented in Table III. The highest amounts of adducts were isolated when H2BP was metabolized in the presence of RSVM and 20:4. The data in Table III show that in the presence of NADPH, MC-induced RLM oxidizes H2BP to a greater extent than PB-induced RLM and noninduced RLM. The difference in the amounts of adducts formed in the presence of RSVM and RLM may be due to differences in the levels of epoxide hydrolase activity. H2BP-epoxide has been shown to be a substrate for epoxide hydrolase and the specific and total activities of epoxide hydrolase are higher in RLM than in RSVM (36, 27). These data demonstrate that the extent of epoxidation of dihydroaromatic hydrocarbons by PGH synthase can be comparable to that catalyzed by classical drug metabolizing enzymes. Although identical protein and substrate concentrations were employed in these in vitro experiments, it is not possible to extrapolate the results to the in vivo situation. Comparative studies intended to quantitate the relative contributions of PGH synthase and mixed function oxidases to xenobiotic metabolism must be performed in cellular preparations derived from target tissues.
Detector sensitivity mNADPH with poly(G) (1 mg/ml). Incubation conditions are for 30 min at 37 °C. HPLC conditions are the same as in Fig. 6. Incubations contained 0.5 mM NADPH and poly(G) with (-)-BP-7,8-diol Diolepoxide 2 90.0 (+)-BP-7,8-diol Diolepoxide 2 11.0 (-)-trans Diolepoxide 1 48.5 (+)-Diolepoxide 2 39.0

 --- Table II ---

Comparison to literature precedents of the stereochemistry of (+)-BP-7,8-diol epoxidation by MC-induced RLM determined by the poly(G) method

Poly(G) adducts isolated from incubation of (+)-BP-7,8-diol with poly(G). The reactions were carried out for 30 min at 37 °C. HPLC conditions are the same as in Fig. 6. Incubations contained 0.5 mM NADPH and poly(G). The reactions were carried out for 30 min at 37 °C. HPLC conditions are the same as in Fig. 1.

--- Table III ---

Relative percentages of (-)-cis and (+)-cis guanosine adducts obtained in vitro from H₂BP with various microsomal preparations

The adducts are quantitated from their absorption at 344 nm. trans Diolepoxides formed in trace amounts in the presence of RLM. The numbers given are average of duplicates ± error.

--- Discussion ---

We have utilized poly(G) as a chiral nucleophile to trap enantiomeric epoxides generated during the oxidative metabolism of H₂BP. When racemic H₂BP-epoxide is reacted with poly(G) and the reisolated nucleic acid hydrolyzed, four nucleoside adducts are isolated. Chemical and spectral evidence indicates that they are diastereomers formed by the cis and trans addition of the exocyclic amino group of guanine to the benzylic carbon of the enantiomeric epoxides. NMR and CD spectroscopy suggest that the first pair of compounds eluting from the reversed phase column (A and B) are trans and the second pair (C and D) cis adducts. When diolepoxides are reacted with poly(G), analogous adducts are formed (38, 39, 44). The principal difference between the adducts formed from H₂BP-epoxide and those formed from diolepoxides is the ratio of cis to trans adducts. The major adducts from H₂BP-epoxide are the cis adducts, whereas the major adducts from the diolepoxides are the trans adducts (38, 44). This may be due to differences in the conformation of the tetrahydrobenzo ring of H₂BP-epoxide relative to the diolepoxides (51).

Kinoshita et al. (52) have recently characterized the deoxynucleoside adducts formed by the reaction of (+)-H₂BP-epoxide with calf thymus DNA. The major adducts arise by the addition of the exocyclic amino group of guanine to the benzylic epoxide carbon. All four cis and trans adducts are formed and the adducts from opposite enantiomers are formed...
in roughly equivalent amounts (52). The spectral properties of the individual deoxyguanosine adducts are very similar to those of the guanosine adducts and the relative order of elution from the reversed phase HPLC column is identical. In addition, the cis-deoxyguanosine adducts predominate (52). Thus, the reaction of H2BP-epoxide with the guanine residues of DNA appears to be very similar to its reaction with poly(G) even though DNA has more extensive secondary structure than poly(G).

The chiral nucleophile trapping approach has been used by others to elucidate the stereochemistry of the formation of polycyclic hydrocarbon epoxides (53, 54). Nonenzymatic reaction of exogenous glutathione has been utilized to determine the stereoselectivity of the epoxidation, by a reconstituted mixed function oxidase, of BP and benzo[a]anthracene (53, 54). Diastereomeric glutathione conjugates of BP-4,5-oxide and benzo[a]anthracene-5,6- and 8,9-oxides were separated on HPLC and quantitated in order to estimate the enantionicomic composition of the epoxides generated. In addition, the diastereomeric composition of the glutathione adducts has revealed the regiochemistry and stereochemistry of the conjugation of BP-4,5-oxide by purified glutathione transferases (55).

An advantage of using poly(G) as the nucleophilic trapping agent is that it can be used with impure enzyme preparations. The polymeric adducts can be isolated and purified prior to hydrolysis to the nucleoside adducts, thereby removing contaminants present in the enzyme preparation. Furthermore, poly(G) is the most reactive of the synthetic polynucleotides toward polycyclic hydrocarbon epoxides (56). A major potential drawback of its use is that its secondary structure may cause preferential reaction with one of the enantiomers of a racemic mixture. This would compromise its utility for quantitative studies of stereoselective epoxide generation. We have not observed such preferential reactivity in our previous studies (38, 39) but as a control we have quantitated the enantiomeric composition of the epoxides generated from (+)-BP-7,8-diol by MC-induced RLM. Previous studies have shown that this system generates enantiomers of diol epoxides 1 and 2 with 9S,10R absolute configuration in high optical purity (16, 17). The data in Table II indicate that these two enantiomers are practically the only products detected in the incubations. This demonstrates that the poly(G) trapping method may be a useful general technique with which to define the stereochemistry of the enzymatic generation of chiral electrophiles capable of diffusing into solution.

The stereoselectivity of H2BP epoxidation by PGH synthase and cytochrome P-450 is very similar to the stereoselectivity of BP-7,8-diol epoxidation by the same enzymes (16, 17, 38, 50). Both enantiomers of H2BP-epoxide are formed in equal amounts from H2BP by PGH synthase implying that there is no stereoselectivity in the introduction of the epoxide oxygen. In contrast, a high degree of stereoselectivity is observed in the NADPH-dependent epoxidation of H2BP by microsomal preparations from control, PB-, and MC-treated rats. From 62-88% of the cis adducts are the (−)-diastereomer (adduct C). The degree of stereoselectivity of epoxidation by cytochrome P-450 appears to be slightly lower for H2BP than for BP-7,8-diol.

Jerina et al. (57) have recently proposed a model for the orientation of polycyclic hydrocarbons at the active site of cytochrome P-450c with respect to the locus of oxygen introduction. When this model is applied to H2BP, it predicts the preferential generation of H2BP-epoxide with 9S,10R absolute configuration. Since MC-induced RLM contain >70% cytochrome P-450c (58), we can tentatively assign the absolute stereochemistry of the major trans and cis adducts and hence the minor adducts as well, based on Jerina's model. The tentative assignments are summarized in Scheme 1.

The dramatic differences in the stereoselectivity of epoxidation of H2BP may be indicative of differences in the mechanisms of epoxidation by cytochrome P-450 and PGH synthase. Oxygen insertion by cytochrome P-450 is believed to occur from a heme iron-oxo complex (59). Although epoxidation may be stepwise, the intermediates do not appear to diffuse away from the active site of the protein (60). The detailed mechanism of epoxidation by PGH synthase is not known. However, the source of the oxygen, stereoselectivity, hydroperoxide specificity, and sensitivity to antioxidants are analogous to the same features of epoxidations catalyzed by hematin in the presence of detergent (61). Hematin-catalyzed epoxidation appears to involve peroxo radicals derived from the unsaturated moiety of the hydroperoxide as oxidizing agent (61). If the mechanism of the PGH synthase-catalyzed epoxidation is similar, the polycyclic hydrocarbon substrate may be in a region removed from the heme prosthetic group or even in the bulk phase. As a result, the approach of the epoxidizing agent (the peroxo radical) may not be restricted to either of the enantiotopic faces, leading to nonstereoselective oxidation.

The stereoselectivity of enzymatic reactions is a reflection of the orientation of the substrate at the active site and provides information about the chirality of the active site and/or the mobility of the substrate. This is graphically illustrated by our comparison of the stereoselectivity of epoxidation of H2BP by PGH synthase and cytochrome P-450. Since these enzymes can be differentiated on the basis of their protein composition (62), substrate specificity (19), inhibitor sensitivity (21, 63), and mechanism (23, 64), our findings provide an additional line of evidence which indicates that these two xenobiotic-metabolizing enzymes are complementary.

The present report demonstrates the utility of poly(G) trapping as a method for determination of the enantiomeric composition of enzymatically generated chiral electrophiles. Since enantiomeric electrophiles can exhibit significant differences in chemical reactivity and biological activity, information about the major enantiomeric metabolites of chiral or prochiral carcinogens can provide important clues to the identity of ultimate carcinogens. Conversely, it may also help explain why certain analogs of known carcinogens are not carcinogenic.

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A Panthananickal, P Weller and L J Marnett

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