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Kinetic Determinants of Benzo[a]pyrene Metabolism to Dihydrodiol Epoxides by 3-Methylcholanthrene-Induced Rat Liver Microsomes

GABRIELA M. KELLER, CHRIS R. TURNER, AND COLIN R. JEFCOATE[1]

Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin 53706

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SUMMARY

Kinetics of benzo[*a*]pyrene (BP) metabolism by 3-methylcholanthrene-induced rat liver microsomes has been determined in conjunction with covalent modification of DNA and formation of (+)-*trans*-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (DE). The prime restraints on further metabolism of 7,8-dihydrodiol to DE (measured as tetrol or DE-dG DNA adduct) were (a) competition between BP and 7,8-dihydrodiol for cytochrome P-450 and (b) inhibition of monooxygenase activity by BP quinones. Removal of BP quinones by glucuronidation [addition of uridine diphosphoglucuronic acid (UDPGA)] stimulated DE formation 5-fold in the absence of DNA. In the presence of DNA, formation of tetrol and DE-dG adducts followed the same time course (DE-dG/tetrol = 0.10), and both were stimulated up to 3-fold by UDPGA. In the presence of UDPGA, the rate of 7,8-dihydrodiol metabolism was comparable to that expected from competition between BP and 7,8-dihydrodiol, whereas, in the absence of UDPGA, it was only 5% of the unihibited rate even at low residual BP concentrations. Glucuronidation of 7,8-dihydrodiol was not detectable. Tetrol and DE-dG formation was not appreciable until more than 80% of BP had been metabolized. DNA adducts derived from 9-phenol-4,5-oxide appeared earlier than DE-dG adducts. This metabolic pathway is apparently less susceptible to inhibition from BP and BP metabolites. BP quinones are conjugated as glucuronides *in vivo* [*Cancer Res.* 41:951–957 (1980)], and thus a potential inhibitor of BP and 7,8-dihydrodiol metabolism is removed.

INTRODUCTION

Substantial evidence now implicates bay-region DE[2] as responsible for the carcinogenic activity of PAH (1). BP typifies this class of molecules in that the bay-region DE (+)-anti-*trans*-7,8-diol-9,10-epoxide [(+)-anti-DE)] is a far more potent transforming agent in mammalian cells than all other metabolites. Stereospecificity of the transformation process is indicated by the lack of activity of the remaining three DE enantiomers. Comparable transformation activity is shown only by (−)-7,8-diol, which is the immediate precursor (2–4). (+)-Anti-DE modifies DNA principally via electrophilic addition to positions N-2 (5), N-7 (6), and O-6 (7) of dG but also forms adducts with dA and dC (8). Such dG adducts have been detected in cells which are transformed by (+)-anti-DE (9). Other important macromolecules, such as RNA, histones, and non-histone proteins, are modified by (+)-anti-DE, and these interactions may contribute to the expression of transformation (10, 11).

Activation of the parent PAH depends both on the intrinsic activity and on the regio- and stereospecificity of microsomal cytochrome P-450 and expoxide hydratase. Thus BP is first metabolized to a mixture of oxides, quinones, and phenols, including 7,8-oxide (4). The latter is selectively hydrated by epoxide hydratase to (−)-7,8-diol and then is further monooxygenated to anti- and syn-DE (12). The proportion of 7,8-oxide formed in the primary attack and the ratio of anti- to syn-DE is highly

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[2] The abbreviations used are: DE, diol epoxide, either or both of the diastereomeric 9,10-epoxides derived from *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene in which the epoxide is *cis* (syn) or *trans* (anti) to the benzylic 7-hydroxyl group; PAH, polycyclic aromatic hydrocarbon; BP, benzo[*a*]pyrene; UDPGA, uridine diphosphoglucuronic acid; Me_2SO, dimethyl sulfoxide; HPLC, high-pressure liquid chromatography; MC, 3-methylcholanthrene; 7,8-oxide, 7,8-epoxy-7,8-dihydrobenzo[*a*]pyrene; 7,8-diol, *trans*-7,9-dihydroxy-7,8-dihydrobenzo[*a*]pyrene; 9,10 diol, *trans*-9,10-dihydroxy-9,10-dihydrobenzo[*a*]pyrene; 3-phenol, 3-hydroxybenzo[*a*]pyrene; 7-phenol, 7-hydroxybenzo[*a*]pyrene; 9-phenol, 9-hydroxybenzo[*a*]pyrene; tetrol, 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; anti-1-tetrol or 7,10/8,9-tetrol, *r*7,*t*8-dihydroxy-*t*9,*c*10-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; anti-2-tetrol, *r*7,*t*8-dihydroxy-*t*9,*t*10-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; syn-1-tetrol, *r*7,*t*8-dihydroxy-*c*9,*t*10-dihydroxy-7,-8,9,10-tetrahydrobenzo[*a*]pyrene; syn-2-tetrol, *r*7,*t*8-dihydroxy-*c*9,*c*10-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; DE-2-dG, N^2-(7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyren-10-yl) deoxyguanosine; 9-PO, 9-phenol-4,5 oxide.

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variable and depends on the form(s) of cytochrome P-450 catalyzing the reaction, which in turn depends upon species, tissue, and exposure of animals to inducing chemicals (4).

The activation sequence is opposed by a set of inactivation reactions, both enzymatic and non-enzymatic. Formation of (−)-7,8-diol from 7,8-oxide may compete with non-enzymatic rearrangement to 7-phenol (4). Conjugation reactions of 7,8-diol with either UDP-glucuronic acid (glucuronyltransferase) (13) or 3′-phosphoadenosine 5′-phosphoadenosine 5′-phosphosulfate (sulfotransferases) (14) compete with secondary monooxygenation of this diol. Finally, (+)-anti-DE rapidly hydrolyzes to tetrols (12, 15, 16) and, at high levels of glutathione *S*-transferase, can be conjugated by glutathione (17, 18). Thus the ultimate conversion of BP to DNA adduct is determined by the balance of activation and inactivation processes.

A third controlling influence has recently been reported by this laboratory (19). BP quinones are potent inhibitors of the monooxygenation of BP and (−)-7,8-diol. These inhibitors can be removed by addition of UDPGA to microsomal incubations, which results in the conjugation of reduced quinones. This release of inhibition elevates DNA modification by DE derived from BP metabolism by nearly 3-fold (20). Here we show that the effect of UDPGA on DNA modification can be traced to a direct stimulation of DE formation. This stimulatory effect is highly time-dependent, and the kinetics of formation of both DE and DNA adducts are examined here for the first time.

MATERIALS AND METHODS

Chemicals. UDPGA, calf thymus DNA, enzymes for hydrolysis of DNA, and reaction cofactors were purchased from Sigma Chemical Company (St. Louis, Mo.). [G-^{3}H]BP (65 Ci/mmole) and ACS were purchased from Amersham Radiochemicals (Arlington Heights, Ill.). Purification of [^{3}H]BP in hexane was carried out by extraction with Me_2SO and KOH according to the method of Van Cantfort *et al.* (21). Methanol and water for HPLC were purchased from Burdick and Jackson Laboratories, Inc. (Muskegon, Mich.). BP metabolite standards were obtained from the National Cancer Institute Chemical Repository. BP was purchased from Aldrich Chemical Company (Milwaukee, Wisc.).

Methods. The induction of male Sprague-Dawley rats (60–70 g; Holtzman Company Madison, Wisc.) with MC and the preparation of microsomes were done by techniques previously described (22).

BP metabolism. Standard incubations to metabolize BP contained MC-induced microsomes at a protein concentration of 0.3 mg/ml; 15 μM [^{3}H]BP (5.33 Ci/mmole); reaction cofactors as previously described (20, 22); 2 mM UDPGA (20); and sonicated, denatured calf thymus DNA (1 mg/ml) (20). UDPGA and DNA were added or omitted as indicated under Results. Total oxidation of BP was measured by the method of Van Cantfort (21), modified as follows. A 50-μl reaction sample was added to 0.55 ml of Me_2SO reagent [a mixture of 8.5 ml of Me_2SO, 1.5 ml of 0.1 M KOH, 3.76 ml 0.05 M KPO_4, (pH 7.5)] and extracted with 2 ml of hexane. Radioactivity was counted in both phases. To quantitate BP metabolites by HPLC, 1-ml reaction samples were extracted with 3 ml of ethylacetate/acetone (2:1) containing 0.15 mM dithiothreitol and the organic phase dried as described previously (20). Radioactivity was counted in both phases. The addition of dithiothreitol improved the stability of BP 3-phenol.[3]

HPLC analysis. HPLC was carried out with a DuPont Model 850 chromatograph equipped with a Model 870 pump, an oven, and a Model 860 fixed wavelength UV detector, and connected to a Schoeffel-Kratos FS970 fluorescence detector and a Waters Associates autoinjector (WISP 710B). Absorbance was monitored at 254 nm, 0.05 AUFS, and excitation and emission wavelengths were set at 248 nm and >389 nm on the fluorescence detector. Solvents were degassed and continuously sparged with helium. BP and its primary metabolites, the diols, phenols, and quinones, were separated on a Whatman Partisil PXS 10/25 ODS-2 column at 35° with a flow rate of 0.4 ml/min, starting with a 20-min linear gradient from 60–100% MeOH and holding at 100% MeOH for 35 min. The elution pattern of the primary metabolites was similar to that reported by Gelboin (ref. 4; Fig. 1C) except that BP 3,6- and 6,12-quinones were not separated by this procedure and the retention times were somewhat longer. The BP tetrols were separated from each other and several other secondary BP metabolites on a DuPont Zorbax-TMS column (0.46 × 25 cm) at ambient temperature with a flow rate of 1 ml/min, starting with 40% MeOH isocratic for 12 min, followed by an exponential (Setting −2) gradient from 40% to 70% MeOH over 20 min and an exponential (Setting −9) gradient from 70% to 100% over 10 min and holding at 100% MeOH for 10 min. The isocratic portion separates the secondary metabolites; the first gradient separates the diols from each other and from the phenols and quinones, which are removed from the column together with the BP by the second gradient. The diols were quantitated on both the TMS and the ODS column and served as a cross-reference between them. A partial fluorescence trace of such a chromatogram is shown in Fig. 1C. Separation of the syn-2-tetrol from 9,10-diol was achieved on a DuPont Zorbax C-8 column (0.46 × 25 cm) at 35°, eluted isocratically with 50% MeOH at a flow rate of 1 ml/min (Fig. 1B).

Determination of DNA adducts. DNA from 1-ml reaction samples was extracted and hydrolyzed to nucleosides as described previously (20). Before hydrolysis, 25-μl samples of the DNA solution were taken to determine the amount of [^{3}H]BP bound and to measure the DNA content by the fluorometric assay described by Thomas and Farquhar (23). The presence of UDPGA did not significantly increase the background fluorescence in this assay. The two major DNA adducts formed by MC-induced microsomes were separated both from each other and from the flow-through peak on a Sephadex LH-20 column (1 × 10 cm) by elution with a step-gradient of aqueous MeOH. This method allows the simultaneous analysis of 15–20 DNA samples and is described in detail

[3] G. M. Keller, unpublished observations.

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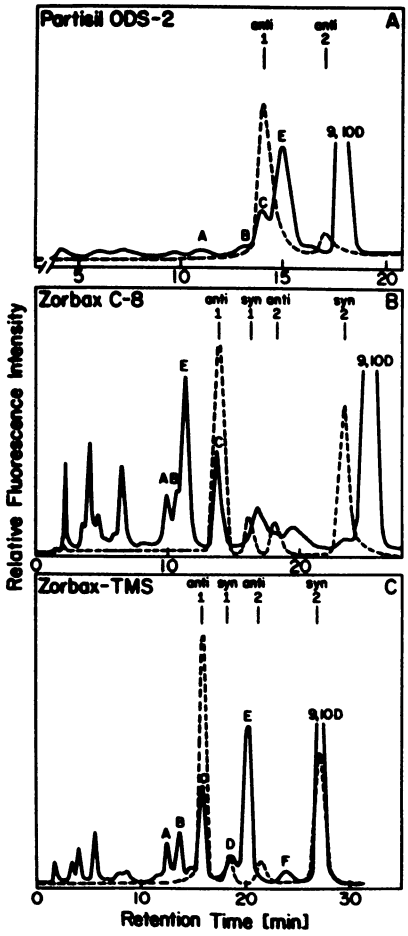

FIG. 1. *Separation of tetrol standards and secondary microsomal BP metabolites by HPLC*

Chromatographic conditions were as described under Materials and Methods. Tetrol standards (– – –) were prepared from synthetic DE by hydrolyzing the anti- and syn-stereoisomers separately in H_2O for 30 min at 37° and extracting with ethylacetate/acetone (2:1) as described under Materials and Methods for BP metabolites. They were chromatographed separately from the BP metabolite sample (——). On the Partisil ODS-2 column, coelution of Peak *C* wtih anti-1-tetrol was verified by analyzing a mixture of unlabeled tetrol standards with a [^{3}H]BP metabolite sample.

elsewhere.[4] The minor adducts (20) were found to contribute less than 10% to the two major adduct peaks.

RESULTS

Quantitation of secondary metabolites. Analysis of the modification of DNA by PAH metabolites requires both the separation of complex mixtures of more polar secondary metabolites and the quantitation of low amounts of these metabolites. Secondary metabolites can be clearly resolved by reverse-phase water/MeOH elution from a DuPont Zorbax-TMS column (Fig. 1C). This less hydrophobic column provides better resolution of more polar products than is obtained with Partisil or Zorbax ODS columns (Fig. 1A). Syn- and anti-tetrols can be separated both from each other and from other secondary metabolites, except that the syn-2-tetrol coelutes with the 9,10-diol. These two metabolites are resolved on a DuPont Zorbax C-8 column (Fig. 1B); however, a poorer separation of other secondary metabolites was obtained on this column. Anti-1-tetrol was the only tetrol which could be quantitated in these experiments. Low amounts of anti-1-tetrol (5 pmoles) have been routinely quantitated by fluorescence during this study with good reproducibility, using the *least* sensitive setting of the detector. The relative fluorescence emission coefficients necessary for quantitation of BP metabolites have been obtained from the ratio of integrated fluorescence intensity (λ_{ex} 248 nm) to recoved ^{3}H counts (Table 1). The relative fluorescence intensities are considerably more dependent on structure than are the relative absorption responses, which varied only 3-fold for the primary BP metabolites. Values for quantitation of primary metabolites and BP by fluorescence and absorbance were in good agreement.

[4] G. M. Keller and C. R. Jefcoate, manuscript submitted for publication.

TABLE 1

Relative fluorescence emission coefficients of BP and its metabolites

HPLC effluent was monitored by fluorescence at λ_{ex} 248 nm λ_{em} > 389 nm. Fractions were collected to quantitate the metabolite in each peak by radioactivity. Fluorescence peak areas were integrated with a Spectra Physics Minigrator.

Compound	Quantity by radioactivity	Fluorescence response	
	pmoles	*AU*[a]	*Au/pmole*
Anti-1-tetrol	4.00	3010	750
9,10-Diol	99.5	12340	124
4,5-Diol	51.9	2540	49
7,8-Diol	63.7	40700	639
9-Phenol	22.6	25110	1110
3-Phenol	41.8	68130	1630
Quinones	73.0	ND[b]	<10
BP	75.0	63810	851

[a] Integrator area units.
[b] Not detectable.

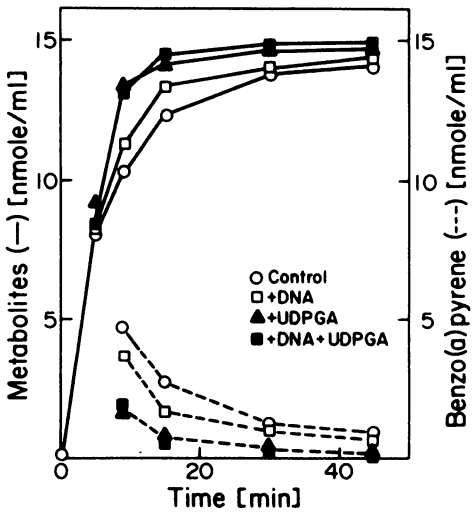

FIG. 2. *Time course of microsomal BP metabolism with or without DNA and UDPGA*

Reaction mixtures contained BP, 15 nmoles/ml, plus one of the following: DNA, 1 mg/ml (□——□); UDPGA, 2 mM (▲——▲); DNA, 1 mg/ml, and UDPGA, 2 mM (■——■); or no addition (control) (○——○). Other conditions were as described under Materials and Methods.

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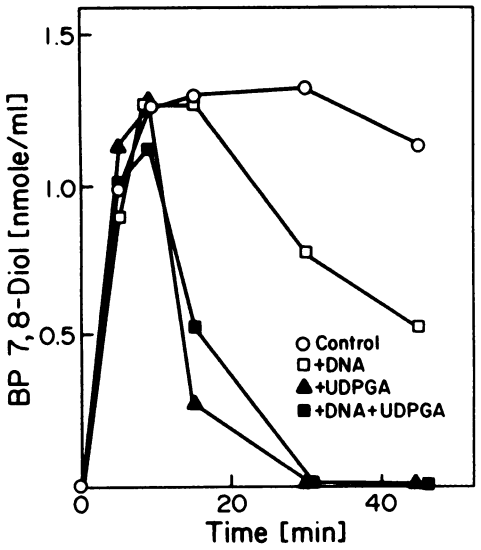

FIG. 3. *Production of BP 7,8-diol during microsomal BP metabolism with or without DNA and UDPGA*
Symbols and conditions are as described for Fig. 2.

Formation of 7,8-diol. Metabolism of BP (15 μM) by MC-induced microsomes (0.3 mg/ml) is linear for about 5 min and then slows appreciably (Fig. 2). Earlier time points had been included in preliminary experiments (data not shown). This decrease in rate is prevented by the addition of UDPGA when linearity is maintained until approximately 80% of the BP has been metabolized. The addition of DNA (1 mg/ml) also limited the late decline in BP metabolism while exerting no effect in the presence of UDPGA. Neither UDPGA nor DNA affected the initial rate of BP metabolism.

Formation of 7,8-diol represents 12% of total primary metabolism of BP. The concentration of 7,8-diol is relatively insensitive to the presence of UDPGA and DNA from 5 to 9 min of reaction (Fig. 3), and differs by only 20% (1.0–1.2 μM) between each set of conditions. However, during the same interval the rate of BP metabolism is doubled by the presence of UDPGA (control 0.55 nmole/min, UDPGA 1.05 nmoles/min). At this time, [7,8-diol] has apparently reached a near steady-state and the effect of UDPGA and DNA is seen only in the length of time that this condition persists. In the control incubation, the steady-state continues for at least 20 min, whereas in the presence of UDPGA (±DNA), a precipitious decline in [7,8-diol] begins after 9 min. The steady-state is maintained for a further 6 min in the presence of DNA without UDPGA. The effects of DNA alone on primary and secondary BP metabolism will be the subject of a separate publication.[4] Treatment with β-glucuronidase did not release significant amounts of 7,8-diol either with BP or 7,8-diol as the substrate in microsomal incubations. Therefore, the effect of UDPGA on 7,8-diol resides almost entirely in stimulating monooxygenation. Glucuronidation of 7,8-diol in microsomal incubations has been reported (13). However, the rate of 7,8-diol glucuronidation was less than 10% of the rate of 7,8-diol metabolism via monooxygenation (19). Glucuronidation of 7,8-diol was also minimal in MC-induced hepatocytes (24) and in rats that received injections or infusions of BP (25, 26).

Two factors contribute to the early loss of steady-state conditions for 7,8-diol in the presence of UDPGA. First, the rate of formation of 7,8-diol declines earlier as [BP] declines below a saturating concentration. Second, the rate of tetrol formation is increased 5-fold by the presence of UDPGA (Fig. 4A), but only after 5 min of reaction. Indeed, up to 5 min there is negligible monooxygenation of 7,8-diol, even though the average [7,8-diol] during this period (0.5 μM) equals the apparent K_m for microsomal monooxygenation of 7,8-diol in absence of inhibitors (19).

Formation of diol expoxide. Competition between 7,8-diol and BP for the active site of cytochrome P-450 should affect both the onset of DE formation and the extent of the stimulatory effect of UDPGA. At 5 min, the

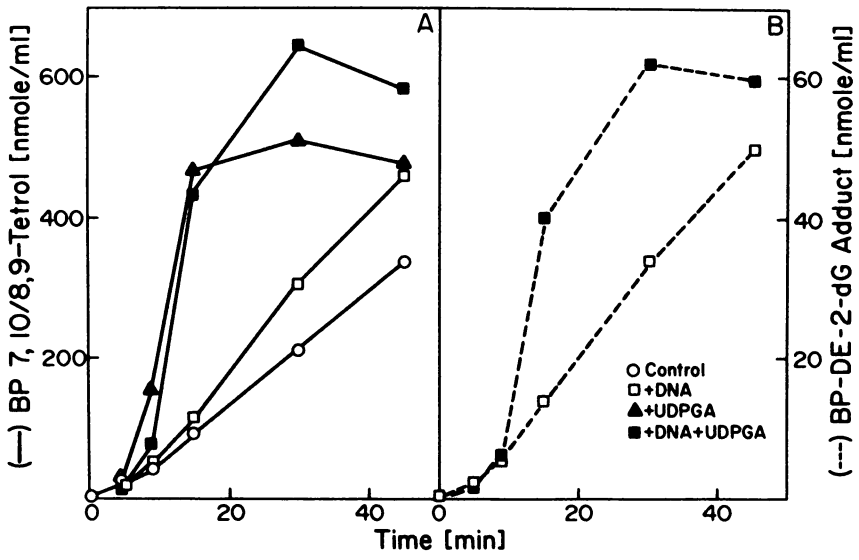

FIG. 4. *Production of BP 7,10/8,9-tetrol (A) and DE-2-dG adduct (B) during microsomal BP metabolism with or without and DNA and UDPGA*
Symbols and conditions are as described for Fig. 2

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TABLE 2

Control of tetrol formation by BP, UDPGA, and DNA

Data used for calculation are represented by the 9- and 15-min points in Figs. 2, 3, and 4.

Condition	[7,8-diol]	[BP]	[7,8-diol]/[BP]	Rate of metabolism		Tetrol formed
				BP	7,8-diol	
	μM	*μM*		*pmoles/min/mg*		*pmoles/min/mg*
Control	1.3	4.8	0.27	1200[a]	140[a]	28
+ DNA	1.3	3.8	0.34	1200[a]	140[a]	33
+ UDPGA	1.3	1.6	0.81	400	580[b]	170
+ DNA + UDPGA	1.13	1.8	0.63	720	420[b]	200

[a] Twelve per cent of the BP metabolized appears as 7,8-diol. A steady state is maintained for [7,8-diol] from 9 to 15 min. Thus the rate of 7,8-diol formation from BP must equal the rate of 7,8-diol metabolism.

[b] The rate of 7,8-diol metabolism is the sum of the decrease in [7,8-diol] per minute and 12% of the rate of BP metabolism between 9 and 15 min. The rate of metabolism exceeds the rate of formation of 7,8-diol.

ratio of [7,8-diol]/[BP] is about 0.15 in all incubations, and at this point BP is substantially more effective than 7,8-diol as a substrate for cytochrome P-450 even though the K_m for BP is about twice that of 7,8-diol. At 9 min, [7,8-diol] has risen only 20%–30% and is essentially the same in each incubation but, in the presence of UDPGA, [7,8-diol]/[BP] has risen 3 times and tetrol formation 6 times (Table 2). After 15 min in the absence of UDPGA, the [7,8-diol]/[BP] ratio increases several-fold to a value comparable with that found in the presence of UDPGA at 9 min. In spite of this increase, there is no increase in the rate of DE or tetrol formation. Thus, in the absence of UDPGA, there is a dominant inhibitory factor(s) which does not change from 9 to 15 min. The major difference in products effected by UDPGA is the near-complete (>95%) removal of BP quinones throughout the reaction. Significantly, a steady-state level of BP quinones is maintained in the control incubation between 5 and 15 min (0.9 μM). This concentration of BP quinones inhibits BP metabolism about 30% but decreases 7,8-diol metabolism by 4-fold (19).

In Table 2, calculated rates of 7,8-diol metabolism indicate a 4-fold increase at 9 min in the presence of UDPGA. The calculated rate of 7,8-diol metabolism is 2–5 times the rate of tetrol formation. When 7,8-diol was directly added as the substrate, tetrol formation accounted for only a portion of the metabolized substrate, 50% in the absence and 20% in the presence of UDPGA. The other products of 7,8-diol metabolism were either more water-soluble than tetrols or, unlike tetrols, were glucuronidated.[3]

The time course of formation of DE-2-dG adducts (Fig. 4B) exactly parallels the formation of tetrol in the presence of DNA. Formation of both tetrol and adducts exhibits a 4-fold rate increase after 9 min when UDPGA is present. In absence of UDPGA, essentially the same low *linear* rate of formation of tetrol and DE-2-dG adducts is maintained from 9 to 45 min. In the presence of UDPGA, all BP and 7,8-diol is consumed after 30 min, precluding further formation of DE. After 45 min the increase caused by UDPGA diminishes to about 20%. Thus, if BP is fully metabolized, the UDPGA effect becomes negligible. This result is consistent with an observed absence of 7,8-diol glucuronides in the presence of UDPGA; i.e., eventually the same proportion of BP and 7,8-diol is converted to DE, irrespective of the presence of UDPGA. When 7,8-diol was the substrate in microsomal incubations, UDPGA had no effect on the formation of DE-2-dG adduct (20). In addition, the formation of DE (tetrols) and DE-2-dG adducts always exhibited the same proportionality; i.e., of every 10 DEs formed at this concentration of DNA, one formed a DE-2-dG adduct (Fig. 5). A similar ratio (6–7) was obtained when anti-DE was added directly to a standard incubation mixture of MC-induced microsomes and DNA.

MC-induced microsomes with lower cytochrome P-450 and monooxygenase activity (55%) exhibited a very comparable UDPGA effect on the formation of tetrol or DE-2-dG adducts. Expanding the time scale by 80% to compensate for the slower monooxygenation provided nearly superimposable time courses for both high- and low-activity microsomes in the presence of UDPGA. The steady-state levels of quinones (1 ± 0.1 μM) and 7,8-diol (1.2 ± 0.2 μM) were similar to those found with the more active microsomes, indicating comparable decreases in activity for both formation and disappearance of these primary metabolites.

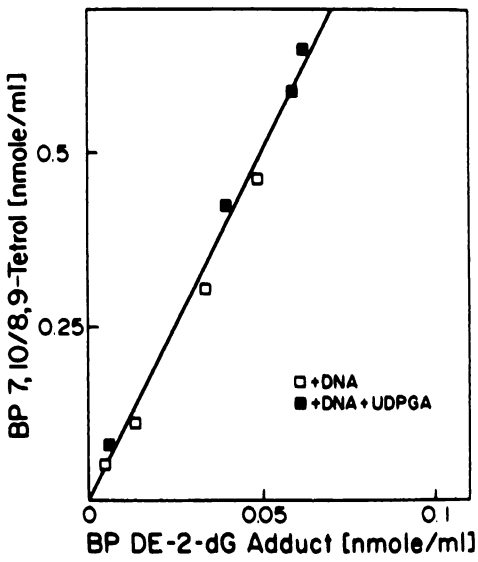

FIG. 5. *Correlation between BP 7,10/8,9-tetrol and DE-2-dG adduct formation during microsomal BP metabolism*

Symbols and conditions are as described for Fig. 2.

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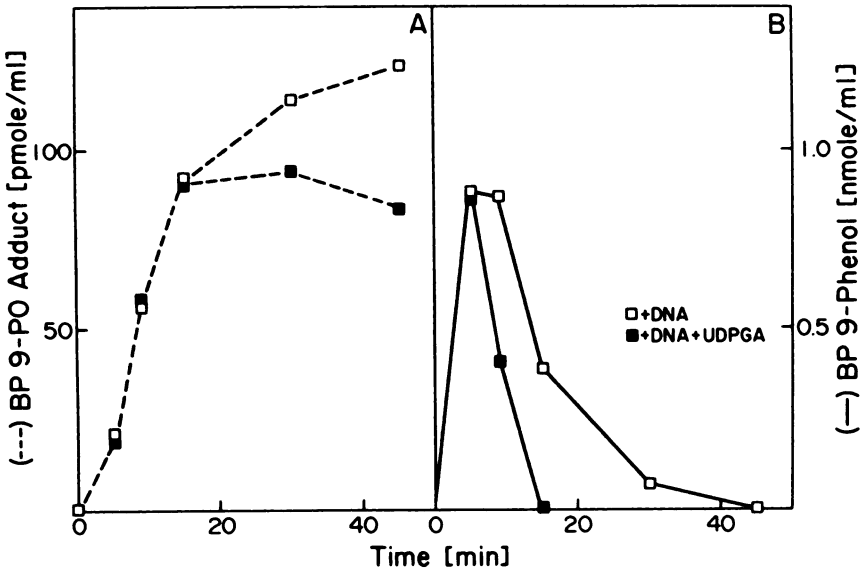

FIG. 6. *Formation of 9-PO DNA adduct (A) and 9-phenol (B) during microsomal BP metabolism in the presence or absence of UDPGA* Symbols and conditions are as described for Fig. 2.

Formation of 9-PO adduct. DNA is also modified by 9-PO (22, 27, 28). The kinetics of formation of these adducts (Fig. 6A) differs from formation of DE-2-dG adducts in two respects. First, the adducts appear at a nearly uniform rate (20 pmoles/min/mg of microsomes) after a lag of only 3 min. Second, adduct formation is relatively insensitive to the presence of UDPGA until a point is reached where 9-phenol is nearly completely consumed. The concentration of 9-phenol at 5 min is independent of UDPGA, but the rate of decline is greatly enhanced by UDPGA (Fig. 6B). Thus, removal of 9-phenol via glucuronidation is much faster than through monooxygenation. Comparison of DNA modification by DE and 9-PO during BP metabolism (Figs. 4B and 6A) indicates that modification by 9-PO is predominant early in the reaction. The ratio of adducts (9-PO/DE) falls from 10 at 9 min to 1.5 at 30 min with UDPGA and 2.5 at 45 min without UDPGA. Results with less active MC-induced microsomes were essentially comparable when the time scale was expanded in proportion to the lower rate of monooxygenation.

DISCUSSION

The metabolism of BP via 7,8-diol to the ultimate carcinogen (+)-anti-7,8-diol-9,10-epoxide is substantially affected by two types of inhibition at both monooxygenation steps: (a) noncompetitive inhihibition by BP quinones (19) and (b) competitive interaction with other BP metabolites at the substrate-binding site of cytochrome P-448. In addition, residual BP competes with 7,8-diol for the cytochrome. The presence of a functioning UDP-glucuronyltransferase, i.e., the addition of UDPGA to microsomal incubations, strongly influences the extent to which these kinetic factors come into play.

In the absence of UDPGA, a steady-state level of 1 μM total BP quinones (1,6-, 3,6-, and 6,12-) is established within 5 min of initiating the reaction. Because of the partially noncompetitive nature, quinones are effective inhibitors at a concentration of 1 μM even when the [BP] is several-fold greater than the K_m (19). Inhibition by other products, many of which bind cytochrome P-450 more weakly than BP, becomes significant only when most of the BP has been metabolized. This concentration of quinones inhibits 7,8-diol metabolism even more effectively as judged by direct measurement (4-fold inhibition). In control incubations (no UDPGA), a 2-fold decrease in [BP] between 9 min and 15 min, while products remain at steady-state levels, does not result in enhanced DE (tetrol) formation. Instead, the constant rate of tetrol formation (9–45 min), which characterizes reaction without UDPGA, is fully consistent with predominant inhibition by the constant steady-state levels of quinones, phenols, and diols. This rate (30 pmoles/min/mg) is only 5% of the uninhibited rate (700 pmoles/min/mg) with the steady-state concentration of 7,8-diol. Competitive inhibition by residual BP should reduce the rate to only 380 pmoles/min/mg. The far lower observed rate supports the crucial role played by other metabolites in the inhibition of 7,8-diol metabolism.

UDPGA radically changes the factors controlling DE (tetrol) formation. Glucuronidation removes quinones almost completely and lowers phenol levels by 2- to 3-fold so that the rate of DE (tetrol) formation becomes dominated by the major remaining inhibitory influence, namely, residual BP. This rate of DE (tetrol) formation in the presence of UDPGA (200 pmoles/min/mg) is relatively close to the calculated activity during partial inhibition by 1.9 μM BP (300 pmoles/min/mg) [assuming K_i (BP) = K_m (BP) = 0.8 μM (19)]. Thus, in the presence of UDPGA, the rate of DE production from BP is largely determined by competition between 7,8-diol and BP.

Addition of DE to position 2 of dG follows exactly the same time course as tetrol formation. We conclude that DE is rapidly transferred to DNA and converted there to either tetrols or adducts. The rapid reaction at DNA is consistent with the report (16) that anti-DE binds to DNA within seconds, resulting in either accelerated tetrol formation or adduct formation. This is also consistent with our observation that the relative formation of tetrol

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to adduct (10:1) is independent of the rate of generation or source of DE (Fig. 5).

The second major DNA adduct formed during BP metabolism is derived from 9-PO (22, 27, 28). These adducts form earlier than DE adducts even though the levels of 9-phenol and 7,8-diol are similar at 5 min in the presence of DNA. Modification of DNA during BP metabolism by 9-PO also differs from modification by DE with respect to insensitivity to UDPGA. The preferred further metabolism of 9-phenol at cytochrome P-450 suggests that it is particularly insensitive to competition from other substrates, especially BP. Possibly 9-phenol is further metabolized without dissociation from cytochrome P-448 and, as a consequence, its secondary metabolism is less sensitive to inhibitory effects.

Quinones are only one class of inhibitors of the primary or secondary metabolism of BP. Other metabolites, particularly 9-phenol, also inhibit both BP and 7,8-diol metabolism. Phenols and reduced quinones are readily glucuronidated *in vivo* (25, 26, 29), and thus potential inhibitors of BP and 7,8-diol metabolism are continually removed. Therefore, DE formation is dependent on the partitioning of metabolism between activation and detoxification pathways. The following factors determine this partitioning: (a) regio- and stereospecificity of P-450(s) for PAH and bay-region diol; (b) relative rates for metabolism of PAH and the product bay-region diol and the respective K_d values for these competing substrates; (c) hydration versus rearrangement rates (or GSH conjugation) of precursor bay-region oxide; and (d) relative rates of oxygenation and conjugation of precursor diol (UDP-glucuronyl transferase or sulfotransferase) [these can be modulated by quinone inhibition (oxygenation) and inhibition of transferases (e.g., by phenol metabolites)]. Any combination of these factors can be altered by external influences. For instance, inducers of cytochrome P-450 not only differ in the pattern of forms of cytochrome P-450 that are induced but also in the induction of expoxide hydrase and conjugating enzymes such as UDP-glucuronyltransferase (29).

Under conditions of *in vivo* exposure or *in vitro* transformation where the dose may be small and fully metabolized, influences on the partitioning of metabolism between carcinogen-yielding and detoxifying pathways may be more important than influences on the level and total reactivity of cytochrome P-450. Indeed, in mouse embryo 10T½ cells, induction of cytochrome P-450 with consequently more rapid metabolism does not result in either increased DNA modification or cell transformation. Induction of PAH metabolism with tetrachlorodibenzo-*p*-dioxin inhibited tumorigenicity of dimethylbenz[*a*]anthracene or BP in mice and prevented binding of BP-DE to epidermal DNA (30). The evaluation of cellular activation of many PAHs whose pathways and ultimate carcinogenic metabolites are known should be facilitated by examining the partitioning of metabolism between activation and detoxification pathways. Our data emphasize the importance of inhibitory effect in determining the production of carcinogenic bay-region oxides.

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Send reprint requests to: Dr. Colin R. Jefcoate, Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisc. 53706.

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