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Hepatic Microsomal Epoxide Hydrase: a Sensitive Radiometric Assay for Hydration of Arene Oxides of Carcinogenic Aromatic Hydrocarbons

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SUMMARY

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A rapid, highly sensitive thin-layer chromatographic assay is described for measurement of epoxide hydrase activity with 11 substrates, including alkene oxides, K-region arene oxides, and non-K-region arene oxides. The highest and lowest specific activities observed were for phenanthrene 9,10-oxide (39 nmoles of product per minute per milligram of protein) and dibenzo[*a*,*h*]anthracene 5,6-oxide (0.4 nmole of product per minute per milligram of protein) with liver microsomes from untreated rats. The most sensitive assay for epoxide hydrase activity was the hydration of benzo[*a*]pyrene 4,5-oxide. Prior treatment of rats with phenobarbital resulted in a 1.7-2.7-fold increase in the rate of hydration for the 11 substrates, whereas 3-methylcholanthrene treatment increased the epoxide hydrase activity approximately 1.3-1.9-fold.

INTRODUCTION

The enzyme epoxide hydrase plays a central role in the metabolic transformation of many olefinic and aromatic drugs and environmental chemicals. Epoxides and arene oxides, formed by the cytochrome P-450 monooxygenase system, are converted into *trans*, vicinal diols through the catalytic action of this microsomal enzyme. In addition to its role in the metabolism and excretion of nonpolar, xenobiotic substrates (1), epoxide hydrase may also protect against the adverse effects of certain drug metabolites. Arene oxides, for example, are potent mutagens toward bacterial and mammalian cells (2-4), have been implicated as hepatotoxic metabolites *in vivo* (5), transform cells in culture (6), and are carcinogenic *in vivo* (7-9). In the absence of further metabolic activation, the dihydrodiols produced from these arene oxides by epoxide hydrase are either weak or inactive as mutagens (7, 8, 10-13) and cell-transforming agents (6).

Despite the wide interest in the possible roles played by epoxide hydrase in altering the metabolism-induced mutagenicity and carcinogenicity of polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene (cf. refs. 12 and 13), relatively little has been done to establish rapid and sensitive assays for the hydration of a variety of polycyclic hydrocarbon arene oxides. The

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present study describes such an assay with 11 alkene and arene oxide substrates. No clear evidence was found to indicate that more than one enzyme was responsible for the activity observed with each of these substrates in microsomes from control as compared with induced animals. The first comparison of the specificity of microsomal epoxide hydrase toward several arene oxides of a single polycyclic aromatic hydrocarbon, benzo[*a*]pyrene, is presented.

MATERIALS AND METHODS

Substrates. Structures of the specifically tritiated alkene and arene oxide substrates and the specific activities at which they were used are given in Table 1. [7-^{3}H]Styrene oxide is the most commonly used substrate (14) for assay of epoxide hydrase. The sample used in the present studies was purchased from New England Nuclear. [7,8-^{3}H]Octene 1,2-oxide was prepared by catalytic reduction of 1,2-epoxy-oct-7-ene in clyclohexane with tritium gas in the presence of 5% palladium on carbon. The product was isolated by vacuum distillation. The structure and purity of the product were established by nuclear magnetic and mass spectra. Hydration of O 1,2-oxide[1] was previously assayed by gas chromatography (15). [2-^{3}H]Naphthalene 1,2-oxide has been used as an epoxide hydrase substrate (16). Syntheses of [3-^{3}H]-phenanthrene 9,10-oxide, [7-^{3}H]benz[*a*]-anthracene 5,6-oxide, and benzo[*a*]pyrene 4,5-, 7,8-, and 9,10-oxides with the tritium label at position 6 have been reported (17). Of these substrates, hydration of P 9,10-oxide has been assayed by high-pressure liquid chromatography (18), and generally labeled BP 4,5-oxide, by thin-layer chromatography (19). Syntheses of [11,12-^{3}H]-benzo[*a*]pyrene 11,12-oxide, [11,12-^{3}H]3-methylcholanthrene 11,12-oxide, and [5,6-^{3}H]dibenz[*a*,*h*]anthracene 5,6-oxide were achieved by cyclization of the tritium-labeled *trans* dihydrodiols (20). Assay of epoxide hydrase activity toward MC 11,12-oxide was previously studied by gas chromatography (21) and high-pressure liquid chromatography (22).

Stock solutions of each substrate were prepared in acetonitrile containing 0.1% concentrated ammonia and stored at −80° prior to use. The presence of the ammonia was essential to prevent acid-catalyzed decomposition of the substrates, particularly the isomerization of non-K-region arene oxides (N 1,2-oxide, BP 7,8-oxide, and BP 9,10-oxide) to phenols.

Enzyme preparations. Immature male Long-Evans rats (50–60 g) were obtained from Blue Spruce Farms, Altamont, N. Y., and were maintained on a commercial diet and water ad libitum. Rats were injected intraperitoneally with sodium phenobarbital (75 mg/kg/day) in NaCl or 3-methylcholanthrene (25 mg/kg/day) in trioctanoin for 4 days. Control rats were injected with either NaCl or trioctanoin. Liver microsomes were prepared in 0.05 M Tris (pH 7.5) containing 1.15% KCl and were washed with 1.15% KCl–10 mM EDTA as previously described (23). The microsomes were suspended in 0.25 M sucrose (20 mg of protein per milliliter) and stored at −90° for 3–7 days prior to use. No significant loss of epoxide hydrase activity was observed for the 11 substrates assayed in this study when microsomes were stored under these conditions. Protein concentrations were determined by the method of Lowry *et al.* (24).

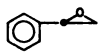
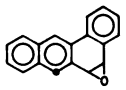
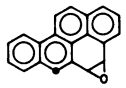
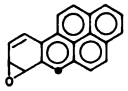
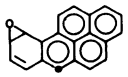
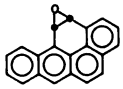
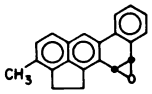
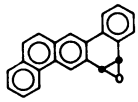
Assay procedures. In order to adequately study 11 substrates simultaneously, a uniform assay procedure was sought which combined speed, sensitivity, and a small incubation volume. Although differential extraction procedures such as those developed for the assay of S 7,8-oxide (14) and N 1,2-oxide (16) have the advantage of high speed, chromatographic assays generally offer increased sensitivity because of lower blanks. Thin-layer chromatography proved to be the method of choice, as a result of the introduction of the 5 × 20 cm LQDF silica gel thin-layer plate by Quantum Industries (25). These four-

[1] The abbreviations used are: O 1,2-oxide, octene 1,2-oxide; P 9,10-oxide, phenanthrene 9,10-oxide; BP 4,5-, 7,8-, 9,10-, and 11,12-oxides, benzo[*a*]pyrene 4,5-, 7,8-, 9,10-, and 11,12-oxides, MC 11,12-oxide, 3-methylcholanthrene 11,12-oxide; N 1,2-oxide, naphthalene 1,2-oxide; S 7,8-oxide, styrene 7,8-oxide; BA 5,6-oxide, benz[*a*]anthracene 5,6-oxide; DBA 5,6-oxide, dibenz[*a*,*h*]anthracene 5,6-oxide.

TABLE 1

Substrates for epoxide hydrase

The dots in each structure indicate the positions of the tritium.

Substrate	Structure	Specific Activity (μCi/μmole)	T.L.C. Solvent System[a]	R_f of Product[b]
[7-^{3}H] Styrene oxide		0.5	A	0.21
[7,8-^{3}H] Octene oxide		2.0	A	0.27
[2-^{3}H] Naphthalene 1,2-oxide		0.9	C	0.22
[3-^{3}H] Phenanthrene 9,10-oxide		7.4	B	0.35
[7-^{3}H] Benzo[a] anthracene 5,6-oxide		9.5	B	0.37
[6-^{3}H] Benzo[a] pyrene 4,5-oxide		5.9	B	0.38
[6-^{3}H] Benzo[a] pyrene 7,8-oxide		8.6	B	0.31
[6-^{3}H] Benzo[a] pyrene 9,10-oxide		5.8	B	0.29
[11,12-^{3}H] Benzo[a] pyrene 11,12-oxide		5.8	B	0.37
[11,12-^{3}H] 3-Methylcholanthrene - 11,12-oxide		3.0	A	0.46
[5,6-^{3}H] Dibenzo[a,h] anthracene - 5,6-oxide		5.9	C	0.25

[a] The thin-layer chromatography (T.L.C.) solvent systems were: A, chloroform–ethyl acetate (8:2); B, benzene–methanol (93:7); C, benzene–chloroform–ethyl acetate (1:1:1).

[b] Chromatographic reference standards of both *cis* and *trans* dihydrodiols used in this study were obtained as described in the references cited for synthesis of the substrates under MATERIALS AND METHODS, and references cited therein. R_F values are those of the *trans* metabolites.

channel plates have a nonabsorbent loading zone to which aqueous solutions can be applied directly. Thus aliquots from four incubations can be applied to the channels of the plate, and all four channels can be developed simultaneously after brief drying of the loading zone in air. Standard chromatographic chambers (4 × 12 × 9 in.,

Brinkmann Instruments) accommodate 10 plates (40 assays) simultaneously. The extremely high efficiency achieved through the use of the Quantum Industries LQDF plate permits thin-layer chromatographic assays which are as rapid as the less sensitive assays based on differential extraction of substrate and product.

The standard incubation mixture consisted of 25 μl of 0.5 M Tris-HCl buffer (pH 8.7 at 37°), 50 μl of water and resuspended microsomes, and 5 μl of substrate solution, in that order, to reach a final volume of 80 μl. The reaction mixture, in the absence of substrate, was brought to room temperature and then incubated at 37° for various times following addition of substrate. Incubation at 37° prior to addition of substrate was found to be unnecessary because of the very small reaction volume. The reaction was terminated by addition of 25 μl of tetrahydrofuran to the incubation mixture, mixing, and then placing the tubes on ice. After the incubation, 35 μl of the mixture (one-third of the sample) were applied to one of the four loading zones on each plate. Application of the sample as a tight band was unnecessary, since this zone of the chromatographic plate is nonabsorbent. Unlabeled carrier diols were added to each chromatogram to permit visualization of the metabolite region under ultraviolet light, except in the case of octene 1,2-glycol, which was visualized with iodine vapor. For incubations under conditions of high conversion of substrate, all the product dihydrodiols, except those from S 7,8-oxide and O 1,2-oxide, which have low extinction coefficients, could be detected under ultraviolet light without added carrier. After development of the plates (see Table 1 for solvent systems), the product bands were localized, the plates were stored in room light for 2 hr, and the gel containing the products was scraped into scintillation vials containing 1.0 ml of methanol (26) and agitated. After addition of 15 ml of Scintisol (Isolab), radioactivity was measured by scintillation spectrometry. The 2-hr period prior to removal of the gel from the plate eliminated the high erroneous background observed because of activation of the plates by ultraviolet light when the gel was counted immediately. Incubations with microsomes which had been boiled for 10 min served as controls and were identical with zero-time incubations, except for incubations with S 7,8-oxide and P 9,10-oxide, for which small but detectable nonenzymatic conversion was observed.

RESULTS

Assay and product analysis. The assay procedure proved highly efficient, in that 100–150 assays were routinely performed by one individual per day. Enzymatic rates were essentially the same when 2–5 μl of acetonitrile, the solvent used to dissolve the substrates, were present in the reaction mixture. Thus the presence of 6% acetonitrile (5 μl) did not appear to inhibit the reaction. All substrates had a broad pH optimum in the range of pH 8.2–9.0 at 37° with microsomes from control and treated animals. Preliminary experiments established that the substrate concentrations used were both saturating and apparently noninhibitory toward catalytic activity. Since recoveries of product dihydrodiols were found to be essentially quantitative (more than 95%) for several selected samples, correction for recovery was unnecessary. For all substrates except the non-K-region arene oxides, only product and unchanged substrate were detected as radioactive bands on the plates. The unstable, non-K-region arene oxides (N 1,2-oxide, BP 7,8-oxide, and BP 9,10-oxide) suffered extensive breakdown to phenols in the course of incubation and analysis. Nonenzymatic rearrangement of the non-K-region arene oxides to phenols did not interfere with the quantitation of the dihydrodiols formed enzymatically, since the phenols had considerably higher R_F values than the dihydrodiols. Spontaneous hydration of K-region arene oxides to dihydrodiols or isomerization to phenols (27) was not substantial under the conditions of the assay.

In order to determine whether any *cis* addition of water occurred during enzymatic hydration of selected arene oxides, carrier amounts of both *cis* and *trans* dihydrodiols were applied to the plates along

with the incubated samples of each of the following substrates: P 9,10-oxide, BA 5,6-oxide, BP 7,8-oxide, and DBA 5,6-oxide. The thin-layer chromatographic separation of the *cis* and *trans* dihydrodiols at positions 7 and 8 of benzo[*a*]pyrene was greatly improved by first saturating the plate with 10% boric acid in methanol and air drying overnight prior to use. The amounts of radioactivity associated with the *cis* dihydrodiol carrier compared to the *trans* isomer ranged from 0% to 2% and were not considered significant. Prior studies on the dihydrodiols from naphthalene (28), phenanthrene (18), and 3-methylcholanthrene (21, 22), produced by epoxide hydrase under conditions in which *cis* isomers would have been detected, also failed to detect significant amounts of *cis* dihydrodiols.

Linearity of product formation with incubation time and protein concentration. Optimal concentrations for the 11 substrates varied over a 20-fold range, from 0.1 to 2.2 mM, depending on the substrate assayed. The substrate concentrations used, for which maximal rates of metabolism were obtained, were as follows: S 7,8-oxide, 1.0 mM; O 1,2-oxide, 1.4 mM; N 1,2-oxide, 2.2 mM; P 9,10-oxide, 0.4 mM; BA 5,6-oxide, 0.3 mM; BP 4,5-oxide, 0.2 mM; BP 7,8-oxide, 0.1 mM; BP 9,10-oxide, 0.2 mM; BP 11,12-oxide, 0.3 mM; MC 11,12-oxide, 0.2 mM; and DBA 5,6-oxide, 0.2 mM. Plots of product formation as a function of incubation time and protein concentration are shown in Figs. 1 and 2, respectively. The only substrate which showed a complicated time curve was N 1,2-oxide (Fig. 1). Although the reaction rate was constant at 2.25 mM N 1,2-oxide for 6 min, sigmoidal plots with a region showing a higher rate were observed at 0.63 and 1.26 mM substrate concentrations. In the case of N 1,2-oxide as substrate, these results are further complicated by the short half-life of this arene oxide in aqueous solution ($t_{1/2}$ = 2–3 min) and possible substrate inhibition of the reaction. Thus, at 0.36 and 1.26 mM N 1,2-oxide, the rate of reaction at earlier times of incubation (up to 2 min) may have been low because of substrate inhibition, but was considerably faster after longer incubation times, probably because the substrate concentration was decreased through metabolism and spontaneous isomerization to the phenol. At high substrate concentrations (2.25 mM), product formation was linear with time (up to at least 6 min), possibly because the rate of the reaction was slowed during the entire incubation by the higher concentration of N 1,2-oxide.

For plots of linearity of diol formation as a function of time (Fig. 1), protein concentrations ranged from a low of 2 μg/80 μl, for P 9,10-oxide, to a high of 200 μg/80 μl, for DBA 5,6-oxide, in order to obtain suitable conversion of substrate. Substrates for which the best linearity was observed, such as O 1,2-oxide, P 9,10-oxide, BP 4,5-oxide, and MC 11,12-oxide, actually displayed linear rates for 2–3 times the 6–8-min periods shown. In contrast, the rates of hydration of BA 5,6-oxide, BP 7,8-oxide, and BP 9,10-oxide were constant for only 1–2 min.

The effect of protein concentration on the rate of diol formation was examined in Fig. 2 with the intention of establishing the upper limit of protein concentration at which linearity was maintained, or a convenient linear range of protein concentration at which the substrate could be assayed. Linearity of product formation as a function of protein concentration for the hydration of O 1,2-oxide, N 1,2-oxide, and BP 7,8-oxide was observed above 150 μg of protein per 80 μl. Hydration of P 9,10-oxide and BA 5,6-oxide was proportional to protein concentration from 0 to 20 μg of protein per 80 μl and was not examined at higher concentrations. The upper limit of linearity of reaction rate ranged from 25 to 100 μg of protein per 80 μl for the remaining substrates. Hydration of S 7,8-oxide was linear with time for at least 15 min, and with protein concentrations as high as 60 μg/80 μl (data not shown).

Effects of prior treatment. Once linear conditions of assay had been established for the 11 substrates, effects of the microsomal inducing agents phenobarbital and 3-methylcholanthrene were examined. The specific activities (nanomoles of product formed per milligram of protein per

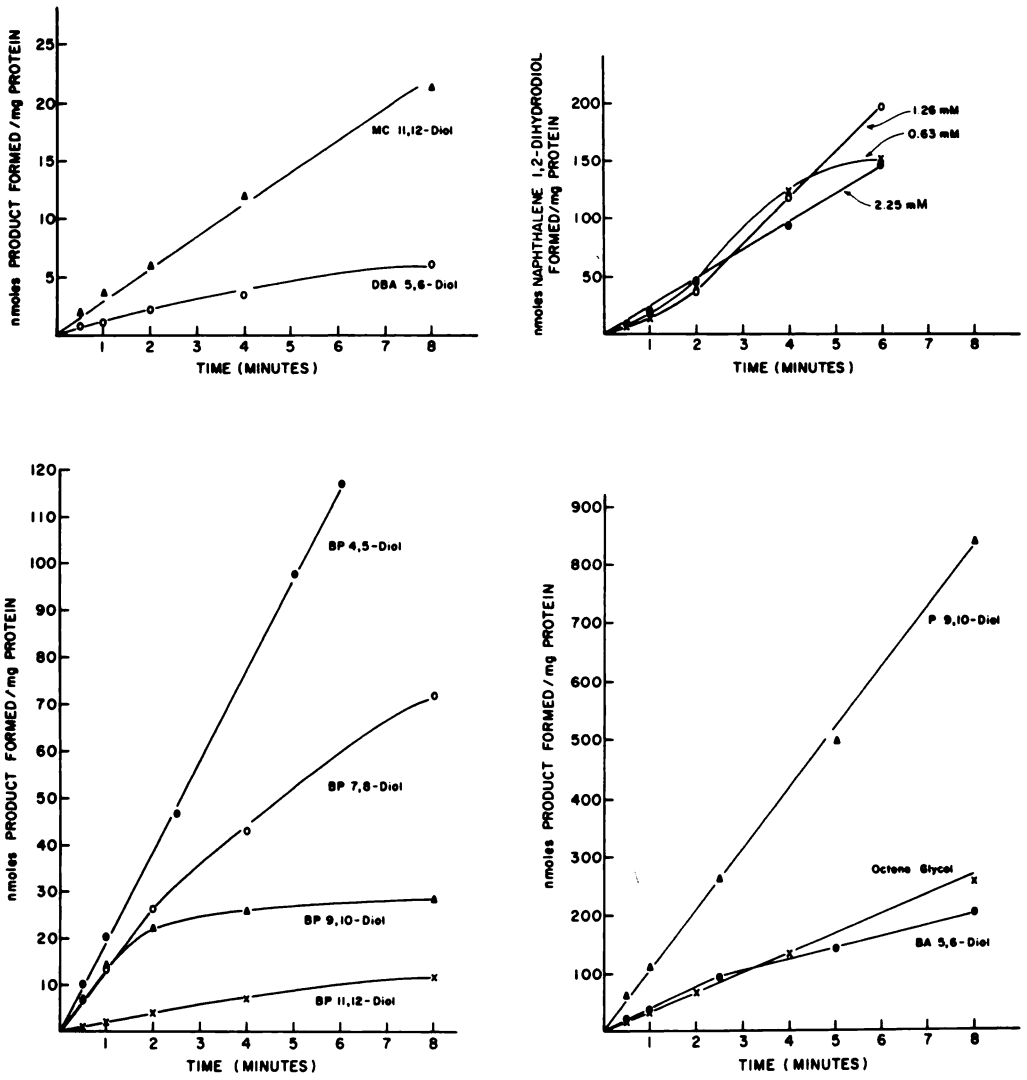


FIG. 1. *Plots of product formation vs. time*

Microsomal protein was obtained from the livers of phenobarbital-treated rats. Substrate concentrations are given under RESULTS. Experimental points are the averages of two or three determinations. The following protein concentrations (per 80-μl incubation) were used: O 1,2-oxide, 20 μg; N 1,2-oxide, 75 μg; P 9,10-oxide, 2 μg; BA 5,6-oxide, 2 μg; BP 4,5-oxide, 10 μg; BP 7,8-oxide, 5 μg; BP 9,10-oxide, 5 μg; BP 11,12-oxide, 50 μg; MC 11,12-oxide, 100 μg; DBA 5,6-oxide, 200 μg.

minute) for liver microsomes from control as well as phenobarbital- and 3-methylcholanthrene-treated animals are presented for all 11 substrates in Table 2. For microsomes from control animals a 100-fold variation in specific activity was observed between the most active and least active substrates: 39.3 and 0.4 nmoles of product per minute per milligram of protein for P 9,10-oxide and DBA 5,6-oxide, respectively. Although P 9,10-oxide is the most active substrate known for epoxide hydrase, considerations of sensitivity of the assay (see below) suggest that other substrates may be more generally useful. Interestingly, MC 11,12-oxide, with the very low specific activity of 1.2 nmoles of product per minute per milligram of protein, has been used as a substrate for two studies of epoxide hydrase activity (21, 22).

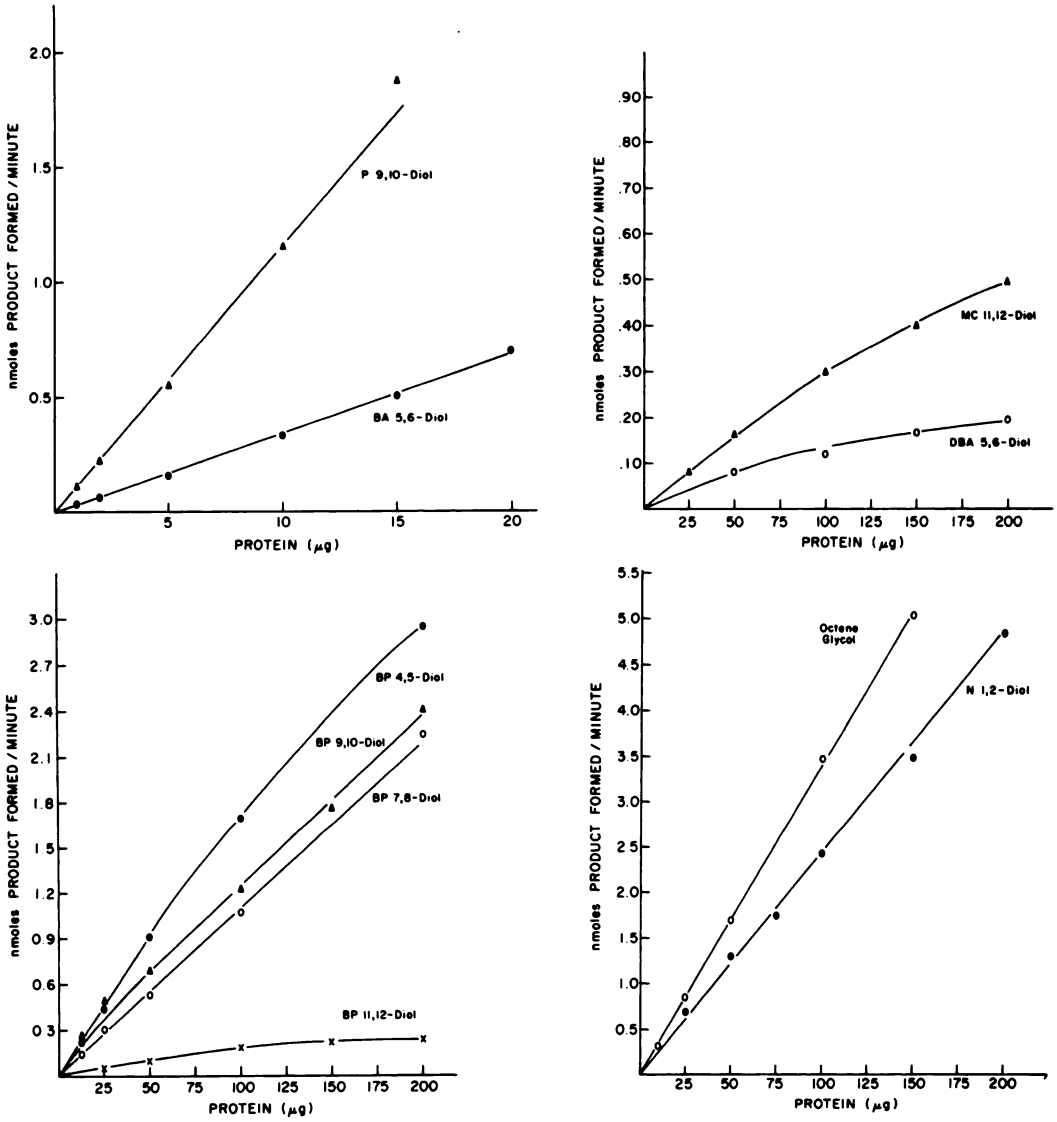


FIG. 2. *Plots of product formation vs. protein concentration*

Protein concentration is expressed as micrograms per 80-μl incubation. Incubation times were 2 min except for O 1,2-oxide and BP 4,5-oxide, for which 5 min were used, and BP 9,10-oxide, for which 1 min was used. Other conditions were the same as in the legend to Fig. 1.

The average induction by phenobarbital was 2.2-fold for the 11 substrates, with BP 7,8-oxide (1.7-fold) and BP 4,5-oxide (2.7-fold) representing the extremes. The average induction by 3-methylcholanthrene with the same substrates was 1.5-fold (range, 1.3–1.9-fold). The similarity in the extent of induction for all 11 substrates with either phenobarbital or 3-methylcholanthrene argues in favor of induction of a single enzyme or a family of enzymes with similar specificity with either inducing agents. In two additional incubation experiments (data not shown), the effect of 3-methylcholanthrene treatment was found to be somewhat variable, with average inductions of 1.2-fold and 1.7-fold for the 11 substrates. The origin of this biological variation is unclear.

Sensitivity of assay. Although none of

TABLE 2

Effects of phenobarbital and 3-methylcholanthrene treatment on rat liver microsomal epoxide hydrase activity

Immature male Long-Evans rats were treated with phenobarbital (75 mg/kg/day) or 3-methylcholanthrene (25 mg/kg/day) for 4 days. Values represent the means ± standard errors for three preparations of microsomes, each of which was prepared from the pooled livers of three rats. All values obtained after treatment with phenobarbital or 3-methylcholanthrene are significantly different from control values ($p < 0.05$). The substrate concentrations are given under RESULTS.

Substrate	Microsomal protein concentration	Incubation time	Expoxide hydrase activity		
			Control	Phenobarbital	3-Methylcholanthrene
	μg/80 μl	*min*	*nmoles product/min/mg protein*		
S 7,8-oxide	50	5	6.3 ± 0.02	13.0 ± 0.7	10.2 ± 0.4
O 1,2-oxide	50	5	13.1 ± 0.2	28.6 ± 1.4	20.2 ± 0.4
N 1,2-oxide	100	2	9.1 ± 0.7	20.8 ± 1.0	15.0 ± 0.3
P 9,10-oxide	10	2	39.3 ± 0.5	89.0 ± 0.9	57.8 ± 2.8
BA 5,6-oxide	20	2	12.4 ± 0.5	29.4 ± 1.4	19.4 ± 0.6
BP 4,5-oxide	15	5	7.2 ± 0.5	19.1 ± 1.0	13.5 ± 0.8
BP 7,8-oxide	25	2	7.8 ± 0.5	15.2 ± 0.5	10.4 ± 0.6
BP 9,10-oxide	25	1	6.4 ± 0.3	15.2 ± 0.6	10.0 ± 1.1
BP 11,12-oxide	50	2	0.8 ± 0.03	1.7 ± 0.02	1.1 ± 0.1
MC 11,12-oxide	50	5	1.2 ± 0.07	2.7 ± 0.09	2.0 ± 0.07
DBA 5,6-oxide	50	2	0.4 ± 0.02	1.0 ± 0.09	0.6 ± 0.02

the present experiments was designed to provide optimum possible sensitivity for each substrate in terms of minimum substrate concentration, maximum protein concentration, and maximum incubation time within linearity, a comparison of the existent data proves most informative. Sensitivity based on the experimental data obtained with liver microsomes from control animals (Table 2) is presented in Table 3. A direct comparison of the number of times product formation exceeded the blank is somewhat misleading, in that some of the substrates, especially P 9,10-oxide and BA 5,6-oxide, were incubated with very little protein. Nonetheless, BP 4,5-oxide appeared to be the most sensitive substrate for the assay. The high sensitivity of the assay for hydration of BP 4,5-oxide was due to a very low blank coupled with an intermediate specific activity for this substrate (Table 2).

DISCUSSION

The present study provides a comparison of hepatic microsomal epoxide hydrase activity toward a range of substrates, including alkene oxides, K-region arene oxides, and non-K-region arene oxides. In addition, the specificity of epoxide hydrase toward all the known arene oxides of a carcinogenic hydrocarbon, benzo[*a*]pyrene, has been measured. More than 20-fold variations in substrate concentrations, 15-fold variations in incubation times, and 100-fold variations in protein concentrations were required in order to achieve linearity of product formation with time of incubation and enzyme concentration for the 11 substrates studied (Table 1).

In the absence of information on comparative specific activities for a range of substrates, compounds such as S 7,8-oxide and MC 11,12-oxide have routinely been used as substrates for determination of epoxide hydrase activity (14, 21, 22). Epoxide hydrase has 84–97% less specific activity toward these substrates than it has toward P 9,10-oxide, presently the most active substrate known for measurement of epoxide hydrase (Table 2). Despite the high specific activity of epoxide hydrase toward P 9,10-oxide, BP 4,5-oxide proved to be the substrate that provided the most sensitive assay, because of a particularly low blank (Table 3). Very low blanks were also observed for BP 11,12-oxide and DBA 5,6-oxide, but low enzymatic activities prevented high sensitivity with these substrates.

TABLE 3

Comparison of sensitivity of assay for all 11 substrates

The concentration of microsomal protein and incubation time for each substrate are described in Table 2. Blank values obtained without enzyme did not significantly increase with time under the conditions of the assay, except in the cases of S 7,8-oxide and P 9,10-oxide.

Substrate	Radioactivity	Radioactivity of product		No. of times blank	Total product formed
		−Microsomes	+Microsomes		
	dpm × 10^{-3}	*dpm*	*dpm*		*pmoles*
S 8,7-oxide	93	302	2,130	7	1,570
O 1,2-oxide	489	2,020	16,100	8	3,180
N 1,2-oxide	306	1,010	4,030	4	1,780
P 9,10-oxide	496	2,620	15,100	6	757
BA 5,6-oxide	421	806	11,100	14	488
BP 4,5-oxide	197	25	7,060	284	533
BP 7,8-oxide	241	2,020	12,600	6	439
BP 9,10-oxide	156	705	2,600	4	182
BP 11,12-oxide	321	71	1,210	17	89
MC 11,12-oxide	100	312	2,220	7	285
DBA 5,6-oxide	237	25	454	18	34

No consistent relationships between structure and specific activity are apparent from the substrates studied. For example, K-region arene oxides ranged from the most active to the least active substrates. Non-K-region arene oxides, such as N 1,2-oxide, and structurally unrelated alkene oxides, such as S 7,8-oxide and O 1,2-oxide, were intermediate in activity. Sterically hindered substrates (BP 11,12-oxide, MC 11,12-oxide, and BP 9,10-oxide) ranged from low to intermediate in activity. For the four arene oxides of benzo[*a*]pyrene which were studied, BP 4,5-, 7,8-, and 9,10-oxides had similar intermediate activities while BP 11,12-oxide had low activity. The latter arene oxide is not a significant metabolite of benzo[*a*]pyrene when microsomes from mouse liver and lung or rat liver are used as the source of the mixed-function oxidase (see ref. 29). Interestingly, the two stereoisomers of the highly mutagenic BP 7,8-diol 9,10-epoxides do not appear to be substrates for purified epoxide hydrase (10, 30).

Variation in induction of enzyme activity toward a range of substrates can be taken as evidence for more than a single enzyme. Since similar induction factors for 11 substrates were observed using microsomes from rats treated with either phenobarbital (average, 2.2-fold) or 3-methylcholanthrene (average, 1.5-fold), no evidence was obtained for multiple forms of epoxide hydrase with different substrate specificities. In addition, an excellent correlation was found between the ratios of the rate of hydration of S 7,8-oxide and the rate of hydration of 10 other epoxide hydrase substrates for each of nine samples of human liver which had been taken at autopsy (31). Thus the use of alkene oxides as well as arene oxides of complex polycyclic aromatic hydrocarbons as substrates appears to measure the same enzyme activity with the rapid, sensitive thin-layer chromatographic assay described. Further studies are required to establish whether epoxide hydrase is a single enzyme in liver tissue or a family of closely related enzymes.

REFERENCES

1. Daly, J. W., Jerina, D. M. & Witkop, B. (1972) *Experientia*, 28, 1129–1149.
2. Ames, B. N., Sims, P. & Grover, P. L. (1972) *Science*, 176, 47–49.
3. Huberman, E., Aspiras, L., Heidelberger, C., Grover, P. L. & Sims, P. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, 68, 3195–3199.
4. Wood, A. W., Goode, R. L., Chang, R. L., Levin, W., Conney, A. H., Yagi, H., Dansette, P. M. & Jerina, D. M. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 3176–3180.
5. Brodie, B. B., Reig, W. D., Cho, A. K., Sipes, G., Krishna, G. & Gillette, J. R. (1971) *Proc.*

Natl. Acad. Sci. U. S. A., **68**, 160–164.

6. Grover, P. L., Sims, P., Huberman, E., Marquardt, H., Kuroki, T. & Heidelberger, C. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, **68**, 1098–1101.
7. Jerina, D. M. & Daly, J. W. (1974) *Science*, **185**, 573–582.
8. Sims, P. & Grover, P. L. (1974) *Adv. Cancer Res.*, **20**, 165–274.
9. Levin, W., Wood, A. W., Yagi, H., Dansette, P. M., Jerina, D. M. & Conney, A. H. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, **73**, 243–247.
10. Wood, A. W., Levin, W., Lu, A. Y. H., Yagi, H., Hernandez, O., Jerina, D. M. & Conney, A. H. (1976) *J. Biol. Chem.*, **251**, 4882–4890.
11. Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Hernandez, O., Dansette, P. M., Jerina, D. M. & Conney, A. H. (1976) *Cancer Res.*, **36**, 3350–3357.
12. Conney, A. H., Wood, A. W., Levin, W., Lu, A. Y. H., Chang, R., Wislocki, P., Goode, R. L., Holder, G. M., Dansette, P. M., Yagi, H. & Jerina, D. M. (1977) in *Biological Reactive Intermediates:* (Jollow, D., Koscis, J., Snyder, R. & Vainio, H., eds.), pp. 335–356, Plenum, New York.
13. Jerina, D. M., Yagi, H., Hernandez, O., Dansette, P. M., Wood, A. W., Levin, W., Chang, R. L., Wislocki, P. G. & Conney, A. H. (1976) in *Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis* (Freudenthal, R. & Jones, P., eds.), pp. 91–113, Raven Press, New York.
14. Oesch, F., Jerina, D. M. & Daly, J. W. (1971) *Biochim. Biophys. Acta*, **227**, 685–691.
15. Oesch, F., Jerina, D. M. & Daly, J. W. (1971) *Arch. Biochem. Biophys.*, **144**, 253–261.
16. Dansette, P. M., Yagi, H., Jerina, D. M., Daly, J. W., Levin, W., Lu, A. Y. H., Kuntzman, R. & Conney, A. H. (1974) *Arch. Biochem. Biophys.*, **164**, 511–517.
17. Yagi, H., Dansette, P. M. & Jerina, D. M. (1976) *J. Labelled Compd. Radiopharm.*, **12**, 127–132.
18. Jerina, D. M., Selander, H., Yagi, H., Wells, M. C., Davey, J. F., Mahadevan, V. & Gibson, D. T. (1976) *J. Am. Chem. Soc.*, **98**, 5988–5996.
19. Gelboin, H. V. & Leutz, J. C. (1975) *Arch. Biochem. Biophys.*, **168**, 722–725.
20. Hernandez, O., Mah, H. D., Dansette, P. M. & Jerina, D. M. (1977) *J. Org. Chem.*, in press.
21. Stoming, T. A. & Bresnick, E. (1973) *Science*, **181**, 951–952.
22. Nesnow, S. & Heidelberger, C. (1975) *Anal. Biochem.*, **67**, 525–530.
23. Lu, A. Y. H. & Levin, W. (1972) *Biochem. Biophys. Res. Commun.*, **46**, 1334–1339.
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
25. Beesley, T. E. (1972) *Am. Lab.*, October, 1–6.
26. Snyder, F. (1964) *Anal. Biochem.*, **9**, 183–196.
27. Bruice, P. Y., Bruice, T. C., Dansette, P. M., Selander, H. G., Yagi, H. & Jerina, D. M. (1976) *J. Am. Chem. Soc.*, **98**, 2965–2973.
28. Jeffrey, A. M., Yeh, H. J. C., Jerina, D. M., Patel, T. R., Davey, J. F. & Gibson, D. T. (1975) *Biochemistry*, **14**, 575–584.
29. Seifried, H. E., Birkett, D. J., Levin, W., Lu, A. Y. H., Conney, A. H. & Jerina, D. M. (1976) *Arch. Biochem. Biophys.*, in press.
30. Thakker, D. R., Yagi, H., Lu, A. Y. H., Levin, W., Conney, A. H. & Jerina, D. M. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, **73**, 3381–3385.
31. Kapitulnik, J., Levin, W., Lu, A. Y. H., Morecki, R., Dansette, P. M., Jerina, D. M. & Conney, A. H. (1976) *Clin. Pharmacol. Ther.*, **20**, 557–564.