[CANCER RESEARCH 48, 1227-1232, March 1, 1988]

Oxidation of the *trans*-3,4-Dihydrodiol Metabolites of the Potent Carcinogen 7,12-Dimethylbenz(*a*)anthracene and Other Benz(*a*)anthracene Derivatives by 3α-Hydroxysteroid-dihydrodiol Dehydrogenase: Effects of Methyl Substitution on Velocity and Stereochemical Course of *trans*-Dihydrodiol Oxidation[1]

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ABSTRACT

The homogeneous 3α-hydroxysteroid-dihydrodiol dehydrogenase of rat liver cytosol catalyzes the NADP-dependent oxidation of a wide variety of polycyclic aromatic *trans*-dihydrodiols and has been implicated in their detoxification (T. E. Smithgall, R. G. Harvey, and T. M. Penning, J. Biol. Chem., *261:* 6184–6191, 1986). This study examined the influence of methyl groups on the velocity and stereochemical course of enzymatic benz(*a*)anthracene (BA) *trans*-dihydrodiol oxidation. The racemic *trans*-3,4-dihydrodiols of BA and 7-methylbenz(*a*)anthracene (7-MBA) were completely consumed by the purified dehydrogenase, indicating that both stereoisomers are substrates. However, 50% of the (±)-*trans*-3,4-dihydrodiols of 12-methylbenz(*a*)anthracene (12-MBA) and 7,12-dimethylbenz(*a*)-anthracene (DMBA) were oxidized, suggesting that only one stereoisomer is utilized in each case. At low substrate concentrations, enzymatic oxidation of the *trans*-3,4-dihydrodiols of BA, 12-MBA, and DMBA followed simple first-order kinetics. By contrast, oxidation of the 3,4-dihydrodiol of 7-MBA was of higher order, due to differences in the rate of oxidation of each stereoisomer. Rate constants estimated for each reaction indicate that the non-bay-region methyl group at position 7 has a greater enhancing effect on the rate of oxidation than the bay-region methyl group at position 12 (10- *versus* 4-fold, respectively). The 3,4-dihydrodiol of DMBA, which possesses both non-bay- and bay-region methyl groups, is oxidized more than 30 times faster than the unmethylated parent hydrocarbon. The absolute stereochemistry of the preferentially oxidized dihydrodiols was assigned by circular dichroism spectrometry. For the 3,4-dihydrodiols of DMBA and 12-MBA, the stereoisomer oxidized has the 3*S*,4*S* configuration. A large negative Cotton effect was observed in the circular dichroism spectrum of the 7-MBA 3,4-dihydrodiol which remained at the end of the rapid phase of oxidation of this racemic substrate, indicating that the dehydrogenase displays partial stereochemical preference for the 3*S*,4*S* enantiomer. These results suggest that methylation of BA at C-7 greatly enhances the oxidation of the 3*S*,4*S*-dihydrodiol, while the presence of a bay-region methyl group at C-12 completely blocks the oxidation of the 3*R*,4*R*-stereoisomer. Rapid, stereoselective oxidation of methylated polycyclic aromatic *trans*-dihydrodiols by this route *in vivo* may significantly influence their carcinogenicity.

Received 6/29/87; revised 11/19/87; accepted 12/1/87.

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[1] This research was supported in part by National Cancer Institute Grant CA 39504 to T. M. P. and by American Cancer Society Grant BC-1321 to R. G. H. Linear regression analysis was performed on the PROPHET Computer system, an NIH-supported research resource.

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[4] The abbreviations and trivial names used are: PAH, polycyclic aromatic hydrocarbons; BA, benz(*a*)anthracene; 7-MBA, 7-methylbenz(*a*)anthracene; 12-MBA, 12-methylbenz(*a*)anthracene; DMBA, 7,12-dimethylbenz(*a*)anthracene; CD, circular dichroism; dihydrodiol dehydrogenase, *trans*-1,2-dihydrobenzene-1,2-diol:dehydrogenase (EC 1.3.1.20); 3α-hydroxysteroid dehydrogenase, 3α-hydroxysteroid:NAD(P) oxidoreductase (EC1.1.1.50); benzenedihydrodiol, (±)-*trans*-1,2-dihydroxy-3,5-cyclohexadiene; androsterone, 5α-androstan-3α-ol-17-one.

INTRODUCTION

PAH[4] are widespread environmental pollutants that have been implicated as etiological agents in human cancer. Metabolic activation of PAH is required for the expression of their carcinogenic effects, and the ultimate carcinogens formed from many of these compounds appear to be bay-region *trans*-dihydrodiol epoxides (1). Activation of benzo(*a*)-pyrene by rat liver microsomes has been studied in detail and is a highly stereoselective process (2). Initial epoxidation of benzo(*a*)pyrene by the cytochrome P-450 mixed-function oxidase system results in the formation of the 7*R*,8*S*-epoxide, which is then enzymatically hydrated to form the *trans*-7*R*,8*R*-dihydrodiol. Secondary cytochrome P-450-mediated epoxidation of the *R*,*R*-dihydrodiol at the 9,10-double bond results primarily in the formation of the (+)-*anti*-diol-epoxide. Of the four diasteriomeric bay-region diol-epoxides chemically possible from benzo(*a*)pyrene, the (+)-*anti*-isomer is by far the most tumorigenic metabolite (3). Thus, the stereochemistry of benzo(*a*)pyrene activation contributes significantly to its carcinogenicity.

The tumorigenic potential of many PAH can be greatly enhanced by the presence of a methyl group on the parent hydrocarbon nucleus (4). For example, BA is only weakly tumorigenic on mouse skin, whereas the structurally related 7-methyl and 7,12-dimethyl derivatives of this compound show greatly enhanced carcinogenicity (5). A similar phenomenon has been observed with chrysene and its 5-methylated derivative. The latter compound rivals benzo(*a*)pyrene in carcinogenicity (6), whereas the unmethylated parent hydrocarbon is almost inactive as a carcinogen (7). Thus, methylation appears to greatly enhance the carcinogenicity of many PAH, particularly when the methyl group is present on the bay region of the molecule (8).

Although enhancement of PAH carcinogenicity by methylation has been well documented, the mechanism responsible for this phenomenon is poorly understood. Methylated PAH appear to undergo carcinogenic activation via bay-region diol-epoxide formation (4), and methyl groups may enhance the reactivity of these diol-epoxides toward DNA. For example, the rate of hydrolysis of the *anti*-diol epoxide of 5-methylchrysene, which contains a bay-region methyl group and is the most tumorigenic of the monomethyl chrysenes (7), is greatly enhanced in the presence of native DNA (9). This diol-epoxide also readily alkylates DNA (9), suggesting that the bay-region methyl group may promote association between DNA and diol-epoxide metabolites. Enhanced reactivity of diol-epoxides of methylated PAH may be related to distortion of the planar conformation of the PAH molecular by the methyl group (10).

In addition to influencing the reactivity of bay-region diol-epoxides, methyl groups could also enhance the carcinogenicity of PAH by altering their metabolism by detoxification enzymes.

One enzyme of interest in this regard is dihydrodiol dehydrogenase (EC 3.1.3.20), which catalyzes the NADP-dependent oxidation of both enantiomeric *trans*-7,8-dihydrodiols of benzo(*a*)pyrene (11) resulting in the formation of the corresponding 7,8-*o*-quinone (12). This reaction can suppress the formation of diol-epoxides, as addition of the purified dehydrogenase significantly reduced the mutagenicity of benzo(*a*)pyrene in the Ames test (13). Although the relative contribution of this enzyme to cellular metabolism of PAH remains to be determined, purification data (14) and immunotitration experiments with polyclonal antibody[5] indicate that dihydrodiol dehydrogenase represents at least 1% of the soluble protein in rat liver. These concentrations suggest that the enzyme could successfully compete with alternative pathways of metabolism for procarcinogenic *trans*-dihydrodiols. The present study demonstrates that methyl groups significantly affect the rate and stereochemical course of BA *trans*-3,4-dihydrodiol oxidation, catalyzed by the homogeneous rat liver dihydrodiol dehydrogenase. The effect of methylation on the rate of enzymatic oxidation is especially remarkable for the 3,4-dihydrodiol of DMBA, which is more than 30-fold faster than that of the unmethylated parent hydrocarbon. Methylation at the bay region also prevents oxidation of the 3*R*,4*R*-dihydrodiol stereoisomer of this potent proximate carcinogen. These findings indicate that dihydrodiol dehydrogenase may significantly influence the stereochemical composition of *trans*-dihydrodiols of methylated PAH available for ultimate carcinogen formation.

MATERIALS AND METHODS

***trans*-Dihydrodiol Synthesis.** Benzenedihydrodiol was synthesized according to our published procedure (15). Other *trans*-dihydrodiols used in this study (Fig. 1) were synthesized according to the methods cited in parentheses: BA 3,4-dihydrodiol (16); 7-MBA 3,4-dihydrodiol (17); 7,12-DMBA 3,4-dihydrodiol (18, 19). A description of the method used to synthesize the 3,4-dihydrodiol of 12-MBA, as well as improved methods for some of the other dihydrodiols will be forthcoming.[6] For a recent review of general methods used in *trans*-dihydrodiol synthesis, see Ref. 20. The purity of the *trans*-dihydrodiols of BA, 7-MBA, 12-MBA, and 7,12-DMBA was confirmed by high performance liquid chromatographic analysis.

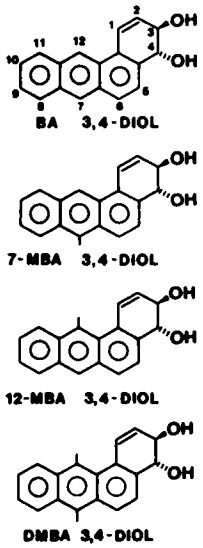

Fig. 1. Structures of *trans*-3*R*,4*R*-dihydrodiols of BA and its methylated derivatives.

Purification of Dihydrodiol Dehydrogenase. Dihydrodiol dehydrogenase was purified to apparent homogeneity from rat liver cytosol using the published procedure (14). In this tissue, dihydrodiol and 3α-hydroxysteroid dehydrogenase activities are catalyzed by the same enzyme. The specific activity of the final preparation was 1.4 μmol androsterone oxidized/min/mg of protein, and 0.3 μmol benzenedihydrodiol oxidized/min/mg protein, when assayed spectrophotometrically at pH 7.0 and 25°C in the presence of either 75 μM androsterone and 2.3 mM NAD or 1.0 mM benzene dihydrodiol and 2.3 mM NADP.

Spectrophotometric Assay of *trans*-Dihydrodiol Oxidation. The initial velocity and end point of enzymatic oxidation of each *trans*-dihydrodiol substrate were determined spectrophotometrically in 1.0 ml of 50 mM glycine buffer, pH 9.0, containing 2.3 mM NADP (Pharmacia/P-L Biochemicals, Piscataway, NJ). Although previous studies have shown that these conditions optimize the rate of *trans*-dihydrodiol oxidation (11) it should be emphasized that these reactions rates are also significant at physiological pH. The dihydrodiols were solubilized in the assay with dimethyl sulfoxide (8% final concentration). Reactions were initiated by addition of the purified enzyme (16–40 μg) and monitored by following the increase in absorbance of the pyridine nucleotide at 340 nm; $\epsilon = 6270\ M^{-1}\ cm^{-1}$ for NADPH. Control incubations showed that no change in absorbance was observed in the absence of enzyme, and that both the dihydrodiol substrate and nucleotide must be present in order for enzymatic oxidation to occur.

Stereochemical Course of *trans*-Dihydrodiol Oxidation. The stereochemical preference of the purified dehydrogenase for each *trans*-dihydrodiol substrate was determined by measuring the CD spectrum of the unreacted dihydrodiol remaining after incubation of the racemate with the purified enzyme. Incubations were conducted in 10 ml of 50 mM glycine buffer, pH 9.0, containing 2.3 mM NADP and 10–25 μM dihydrodiol solubilized in dimethyl sulfoxide. Following addition of the purified enzyme (16–40 μg/ml), the reactions were incubated at 25°C for various time intervals and then terminated by extraction of the dihydrodiols with ethyl acetate (3 × 5-ml aliquots). The organic solvent was removed under reduced pressure, and the resulting residues were chromatographed on 250-μm silica gel thin layer chromatography plates using chloroform:ethyl acetate (1:1) as running solvent. The unreacted diols were visualized under UV and extracted from the silica with ethanol. CD spectra were recorded on an Aviv Model 60DS spectropolarimeter at room temperature using a quartz cell with 1-cm path length. CD spectra are expressed as millidegrees of ellipticity for ethanolic solutions of each diol that absorb approximately 1.0 at their UV wavelength of maximum absorbance.

RESULTS

Initial Velocity of *trans*-Dihydrodiol Oxidation by Dihydrodiol Dehydrogenase. The *trans*-3,4-dihydrodiols of BA, 7-MBA, 12-MBA, and DMBA are readily oxidized by the homogeneous rat liver dihydrodiol dehydrogenase in the presence of NADP at pH 9.0 and 25°C (Table 1). The limited solubility of these compounds prevented direct determination of K_m and V_{max}

Table 1 *Oxidation of the trans-3,4-dihydrodiols of BA and its mono- and dimethylated derivatives by homogeneous dihydrodiol dehydrogenase*

Oxidation of the *trans*-3,4-dihydrodiols of BA, 7-MBA, 12-MBA, and DMBA was monitored spectrophotometrically by measuring the change in absorbance of the pyridine nucleotide at 340 nm under the reaction conditions given. Initial velocities shown are the mean values calculated from at least three independent determinations; in all cases the SE was less than 10% of the mean.

3,4-Dihydrodiol	Concentration (μM)	Initial velocity (nmol/min/mg)	V_{max}/K_m[a]
BA	25	11.4	456
7-MBA	25	22.1	884
12-MBA	10	4.63	463
DMBA	25	46.9	1877

[a] V_{max}/K_m was calculated by dividing v (nmol/min/mg enzyme) by s (μM) and multiplying the result by 1000.

[5] T. E. Smithgall and T. M. Penning, submitted for publication.
[6] R. G. Harvey and C. Cortez, manuscript in preparation.

values. However, the utilization ratio (V_{max}/K_m) can be computed for each dihydrodiol because the reactions were conducted at very low substrate concentrations relative to K_m. Under these conditions, the initial velocities follow first-order kinetics, and the Michaelis-Menten equation simplifies to

$$\frac{v}{s} = \frac{V_{max}}{K_m}$$

(21). Estimates of the utilization ratios obtained in this manner for each *trans*-dihydrodiol substrate (Table 1) are directly comparable to those previously reported for a wide variety of polycyclic aromatic *trans*-dihydrodiols (11). Comparison of these values indicates that the *trans*-3,4-dihydrodiol of DMBA is oxidized very rapidly by the dehydrogenase, as the V_{max}/K_m for this potent proximate carcinogen is almost 4-fold higher than that observed for the unmethylated parent hydrocarbon and almost 6 times higher than that previously reported for the 7,8-dihydrodiol of benzo(*a*)pyrene (11). The utilization ratio observed for the 3,4-dihydrodiol of 7-MBA is also significantly higher than that of the unmethylated BA dihydrodiol, suggesting that the presence of a methyl group at position 7 may enhance the initial rate of oxidation. These results are consistent with earlier studies in which *trans*-dihydrodiols of 5-methylchrysene were shown to be more rapidly oxidized than *trans*-dihydrodiols of the unmethylated parent hydrocarbon (11). Methylated polycyclic aromatic *trans*-dihydrodiols in general may be more readily oxidized by the dehydrogenase.

Determination of the End Point of Enzymatic *trans*-Dihydrodiol Oxidation. To determine whether or not methyl groups alter the stereochemical course of BA *trans*-dihydrodiol oxidation, the end point of oxidation of each racemic dihydrodiol substrate was determined spectrophotometrically. Oxidation of each *trans*-3,4-dihydrodiol substrate was monitored at 340 nm until no further change in absorbance was observed; addition of a second aliquot of enzyme at this time resulted in no further absorbance change, confirming that each reaction had reached completion. Progress curves for these reactions are shown in Fig. 2. For the racemic *trans*-3,4-dihydrodiols of 12-MBA and DMBA, the absorbance changes observed were approximately 50% of the theoretical maximum [calculated from the substrate concentration and the molar extinction coefficient for NAD(P)H, which is formed stoichiometrically during the reaction]. These results suggest that one stereoisomer was completely oxidized in each case; the preferred stereoisomers were later identified by circular dichroism spectropolarimetry (see below). However, the total absorbance changes observed for the 3,4-dihydrodiols of BA and 7-MBA were both approximately 100% of the theoretical maximum, suggesting that both enantiomers of these diols are substrates for the dehydrogenase (Fig. 2*B*).

Pseudo First-Order Analysis of Absorbance Data. Determination of the end point of oxidation of each *trans*-dihydrodiol was conducted at diol concentrations well below K_m. Therefore, these reactions can be regarded as simple, first-order processes, and semilog plots of the fraction of substrate remaining *versus* time should be linear. Absorbance data obtained for all of the *trans*-dihydrodiol substrates were reanalyzed in this manner and resulted in linear semilog plots for the 3,4-dihydrodiols of 12-MBA and DMBA (Fig. 3*A*), as well as for the 3,4-diol of BA (Fig. 3*B*). However, transformation of the absorbance data obtained with the 3,4-dihydrodiol of 7-MBA resulted in a curvilinear semilog plot (Fig. 3*B*), indicative of a higher order reaction consisting of fast and slow components (Fig. 3*C*). This result indicates that although the dehydrogenase oxidized both

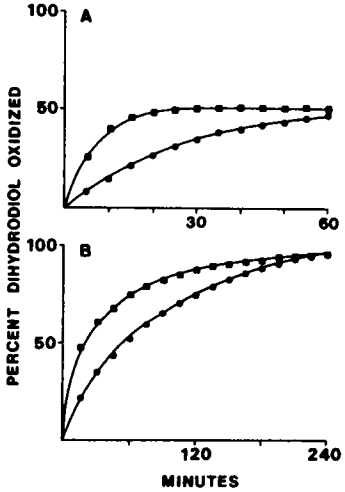

Fig. 2. Progress curves of *trans*-dihydrodiol oxidation. The NADP-dependent oxidation of *trans*-dihydrodiols, catalyzed by dihydrodiol dehydrogenase, was followed spectrophotometrically at 340 nm as described under "Materials and Methods." Substrates were present in the following concentrations: 3,4-dihydrodiols of BA, 7-MBA, and DMBA, 25 μM; 3,4-dihydrodiol of 12-MBA, 10 μM. Results are expressed as percentage of racemic dihydrodiol oxidized, based on the substrate concentration in each reaction and the molar extinction coefficient for NADPH. *A*, oxidation of the 3,4-dihydrodiols of DMBA (■) and 12-MBA (●); *B*, oxidation of the 3,4-dihydrodiols of BA (●) and 7-MBA (■).

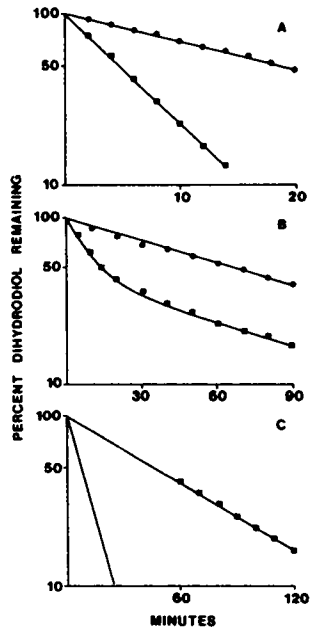

Fig. 3. Pseudo-first-order analysis of enzymatic *trans*-dihydrodiol oxidation. The percentage of each *trans*-dihydrodiol substrate remaining (log scale) at a given time was calculated from the observed end point of each individual reaction according to the simple relationship

$$\%\ \text{of diol remaining} = \frac{(A_{max} - A_t)}{A_{max}} \times 100$$

where A_{max} is the maximum absorbance change observed at the conclusion of the experiment and A_t is the absorbance change observed at a given time point. *A*, pseudo-first-order plots of DMBA (■) and 12-MBA (●) 3,4-dihydrodiol oxidation; *B*, pseudo-first-order plots of BA (●) and 7-MBA (■) 3,4-dihydrodiol oxidation; *C*, pseudo-first-order plots of the fast and slow components of 7-MBA 3,4-dihydrodiol oxidation. Estimates of the fast and slow components of 7-MBA 3,4-diol oxidation were obtained by fitting a regression line to the slow component of the reaction (■); this line was extrapolated to the origin to allow estimation of the change in absorbance due to the slow component during early time points. The estimated absorbance changes were then subtracted from the total absorbance change to yield the contribution of the fast component.

stereoisomers of the *trans*-3,4-dihydrodiol of 7-MBA, one stereoisomer was oxidized more rapidly than the other.

Although V_{max}/K_m data (Table 1) provided an initial estimate of the relative efficiency of enzymatic *trans*-dihydrodiol oxidation and allowed for direct comparison with previously published data (11), these estimates do not take into account differences in stereochemical preference displayed by the dehydrogenase for each racemic *trans*-dihydrodiol substrate. Therefore, pseudo-first-order rate constants were determined to provide a better estimate of the relative efficiency of BA *trans*-dihydrodiol oxidation (Table 2). For the *trans*-3,4-dihydrodiols of BA, 12-MBA, and DMBA, rate constants were estimated directly from the slopes of the lines in Fig. 3 by linear regression analysis. For the 3,4-diol of 7-MBA, which showed biphasic kinetics, rate constants for the fast and slow components of the reaction were calculated based on the assumption that both reactions are first-order processes (appropriate correction for the contribution of the slow component in the calculation of the rate constant for the fast component was made; see the legend to Fig. 3 for details). Comparison of the rate constants obtained in this manner indicates that the 3,4-dihydrodiol of DMBA is oxidized more than 30 times faster by the enzyme than the 3,4-dihydrodiol of BA. The rate constants obtained for the 3,4-dihydrodiols of 7-MBA (fast component) and 12-MBA are also significantly higher than that observed for the 3,4-dihydrodiol of BA (10- and 4-fold, respectively), suggesting that the presence of either methyl group greatly enhances the rate of oxidation of BA non-K-region dihydrodiols. Although both methyl groups probably enhance the rate of oxidation of the 3,4-dihydrodiol of DMBA, differences in the rates of oxidation of the two monomethylated BA 3,4-dihydrodiols suggest that the non-bay-region methyl group (position 7) may have the predominant effect on rate enhancement. In addition, methylation appears to have a progressive influence on the stereochemical course of dehydrogenation. Both stereoisomers of the 3,4-dihydrodiol of BA are oxidized slowly, and apparently at equal rates. However, introduction of a methyl group at position 7 enhances the oxidation of one stereoisomer by almost 10-fold, and the presence of the bay-region methyl group (position 12) appears to block the oxidation of one stereoisomer completely.

Identification of the *trans*-Dihydrodiol Stereoisomers Preferentially Oxidized. In order to identify the stereoisomers preferentially oxidized by dihydrodiol dehydrogenase, large-scale incubations were conducted with each racemic *trans*-dihydrodiol substrate and the purified enzyme. At the end of each reaction, the unoxidized substrate was extracted and isolated by thin layer chromatography, and its CD spectrum was recorded. The length of each incubation was based on the progress of the reaction monitored simultaneously at 340 nm.

The 3,4-dihydrodiols of 12-MBA and DMBA were incubated with the enzyme for 4 h prior to CD spectroscopy of the unreacted dihydrodiol. Both reactions plateaued at 50% of their theoretical absorbance maximum during the first hour of this incubation period. The CD spectra of the unreacted 3,4-dihydrodiols isolated from these reactions are shown in Fig. 4, *A* and *B*. Comparison of these spectra with one published for the optically pure 3*S*,4*S*-dihydrodiol of DMBA (22) indicates that the diols remaining at the end of the reaction have the 3*R*,4*R* conformation. Thus, the enzyme appears to display absolute stereochemical preference for the 3*S*,4*S*-dihydrodiols of both 12-MBA and DMBA.

Although the 3,4-diol of 7-MBA was shown to be completely oxidized by the enzyme based on the total change in absorbance at 340 nm (Fig. 2*B*), pseudo-first-order kinetic analysis suggests that the enzyme oxidizes one stereoisomer faster than the other (Fig. 3*B*). Further support for this conclusion was obtained by incubating this 3,4-dihydrodiol with the purified enzyme until the rapid phase of the reaction was complete (approximately 1 h); a large negative Cotton effect was observed in the CD spectrum of the unreacted isomer isolated from the reaction at this time (Fig. 4*C*). Comparison of this CD spectrum with one published for the optically pure 3*R*,4*R*-dihydrodiol of BA (23) suggests that the 3,4-dihydrodiol of 7-MBA is enriched with

Table 2 *Pseudo-first-order rate constants for the oxidation of the trans-3,4-dihydrodiols of benz[a]anthracene and its methylated derivatives*

Pseudo-first order rate constants for the enzymatic oxidation of the 3,4-dihydrodiols of BA, 12-MBA, and DMBA were estimated directly from the slope of semilog plots of percent dihydrodiol substrate remaining *versus* time (representative plots are shown in Fig. 3, *A* and *B*). The rate constants for the oxidation of the *S,S* and *R,R* enantiomers of the 3,4-dihydrodiol of 7-MBA were determined by regression analysis of the slopes of the two component reactions that contribute to the biphasic semilog plot observed with this substrate (see Fig. 3, *B* and *C*). The absolute stereochemistry of the preferred dihydrodiols was determined by CD spectroscopy (see text and Fig. 4). Rate constants and percentage of conversion values are means of at least three independent determinations; in all cases the SE was less than 10% of the mean.

3,4-Dihydrodiol	Stereoisomer	k (min^{-1} mg^{-1} enzyme)	% of conversion
BA	*S,S* and *R,R*	0.26	112
7-MBA	*S,S*	2.51	108
	R,R	0.26	
12-MBA	*S,S*	0.98	48
DMBA	*S,S*	8.69	47

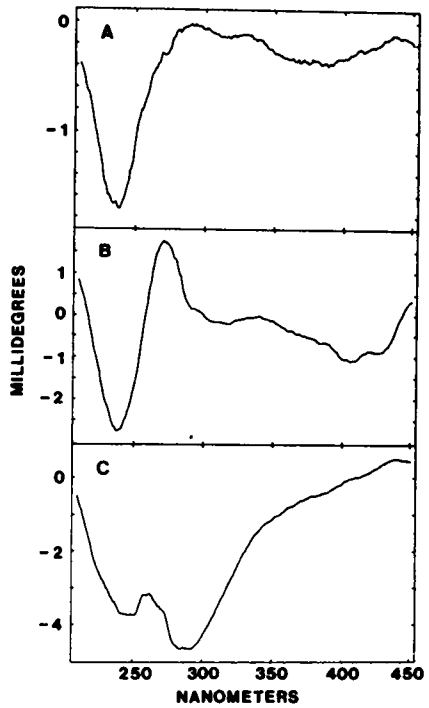

Fig. 4. Identification of the *trans*-dihydrodiol stereoisomers preferentially oxidized by dihydrodiol dehydrogenase. Large-scale enzymatic oxidation reactions containing the 3,4-dihydrodiols of 12-MBA, DMBA, and 7-MBA were conducted as described under "Materials and Methods," and their progress was followed simultaneously at 340 nm. For the dihydrodiols of 12-MBA and DMBA, no further change in absorbance was observed after 4 h. Both reactions were extracted with ethyl acetate at this time, and the unoxidized dihydrodiol substrates were isolated by thin layer chromatography and their CD spectra were recorded. The 3,4-dihydrodiol of 7-MBA was incubated with the dehydrogenase for 1 h prior to extraction; pseudo-first-order analysis (Fig. 3, *B* and *C*) shows that the fast phase of the reaction has reached completion at this point. The unoxidized substrate was isolated by thin layer chromatography and its CD spectrum was recorded. The CD spectra shown were then compared to published spectra for optically pure dihydrodiols of BA and DMBA (22, 23), and they correspond to the 3*R*,4*R*-dihydrodiols of 12-MBA (*A*), DMBA (*B*), and 7-MBA (*C*).

the *R,R*-isomer.[7] Thus, dihydrodiol dehydrogenase displays partial stereochemical preference for the 3*S*,4*S*-dihydrodiol of 7-MBA. As a control, oxidation of the racemic 3,4-dihydrodiol of BA was conducted for 60 min, at which point the reaction was 50% complete. No Cotton effect was observed in the CD spectrum of the unused substrate, suggesting that dihydrodiol dehydrogenase displays no stereochemical preference for either of the stereoisomers of the 3,4-dihydrodiol of BA. This result is consistent with the linear first-order plot (Fig. 3*A*) and the end point of the reaction (100%; Table 2).

DISCUSSION

This report demonstrates that the homogeneous dihydrodiol dehydrogenase of rat liver cytosol catalyzes the NADP-dependent oxidation of the *trans*-3,4-dihydrodiols of BA and several of its methylated, more carcinogenic derivatives. Comparison of pseudo-first-order rate constants for the oxidation of these dihydrodiols shows that methylation greatly enhances the efficiency of oxidation. This effect is especially marked for the 3,4-dihydrodiol of DMBA, which is utilized at more than 30 times the rate of the 3,4-dihydrodiol of BA. We have reported a similar phenomenon for the *trans*-1,2- and 7,8-dihydrodiols of 5-methylchrysene, which are oxidized 9- and 6-fold faster than the 1,2-dihydrodiol of the unmethylated parent hydrocarbon (11).

In addition to rate enhancement, methylation of the parent hydrocarbon also significantly influences the stereochemical course of enzymatic *trans*-dihydrodiol oxidation. Although the dehydrogenase oxidizes both enantiomeric 3,4-dihydrodiols of BA and 7-MBA, the presence of a non-bay-region methyl group at position 7 enhances the rate of oxidation of the 3*S*,4*S*-dihydrodiol by almost 10-fold. In addition, the presence of the bay-region methyl group at position 12 completely blocks the oxidation of the 3*R*,4*R*-dihydrodiol stereoisomer of both 12-MBA and DMBA. Thus, depending on the substrate, dihydrodiol dehydrogenase can display an apparent lack of stereochemical preference (3,4-dihydrodiol of BA), partial stereochemical preference (3,4-dihydrodiol of 7-MBA), or absolute stereochemical preference (3,4-dihydrodiols of 12-MBA and 7,12-DMBA). We have previously reported (11) other *trans*-dihydrodiol substrates for which the enzyme appears to lack a stereochemical preference [7,8-dihydrodiol of benzo(*a*)pyrene] or for which it displays at least partial if not absolute stereochemical preference [*e.g.*, the *trans*-1,2- and 7,8-dihydrodiols of 5-methylchrysene].

The carcinogenic activation of BA (24), 7-MBA (25), and DMBA (26) appears to proceed through the *trans*-3,4-dihydrodiols to bay-region diol-epoxides. Oxidation of these *trans*-dihydrodiol proximate carcinogens by dihydrodiol dehydrogenase to less reactive metabolites could suppress the formation of diol-epoxide ultimate carcinogens. Since the stereochemistry of BA and DMBA activation has been more thoroughly investigated than that of the monomethylated benz(*a*)anthracenes, discussion of the potential contribution of dihydrodiol dehydrogenase to detoxification will be limited to these two compounds.

Formation of *trans*-3,4-dihydrodiols from BA by Sprague-Dawley rat liver microsomes parallels that observed for the *trans*-7,8-dihydrodiols of benzo(*a*)pyrene in terms of stereochemistry, as the predominant stereoisomer formed in each case has the *R,R* configuration (2). However, subsequent metabolism of the enantiomeric BA 3,4-dihydrodiols by cytochrome P-450 is markedly different. Whereas both the 7*R*,8*R* and 7*S*,8*S* dihydrodiols of benzo(*a*)pyrene can be activated to diol-epoxides by rat liver microsomes (27), the 3*R*,4*R*-dihydrodiol of BA is only poorly converted to the corresponding bay-region diol-epoxide, and no detectable diol-epoxides are formed from the 3*S*,4*S*-dihydrodiol (28). Poor conversion to diol-epoxides may partially explain why BA is weakly tumorigenic compared to other PAH, even though chemically synthesized bay-region diol-epoxides of this compound are active mutagens (29) and carcinogens (24). In addition, dihydrodiol dehydrogenase may suppress diol-epoxide formation by competing for the 3*R*,4*R*-dihydrodiol proximate carcinogen formed *in vivo*. Recent identification of a novel *o*-quinone (3,4-diketone) metabolite of BA supports this hypothesis (28), as *o*-quinones have been identified as the products of the enzymatic oxidation of other *trans*-dihydrodiols (12).

Unlike BA, metabolism of DMBA to *trans*-3,4-dihydrodiols appears to be less stereoselective. Liver microsomes from both control and 3-methylcholanthrene-treated Sprague-Dawley rats have been reported to produce 3,4-dihydrodiols of DMBA with a 3*R*,4*R*:3*S*,4*S* ratio of approximately 60:40, in contrast to 90:10 for BA (2). The metabolites of DMBA that bind to DNA of cultured mammalian cells appear to be derived from both *syn*- and *anti*-3,4-diol-1,2-epoxides (30), but it is unclear at this time whether either or both of the enantiomeric *trans*-3,4-dihydrodiols of DMBA give rise to these diol-epoxides. Of these and all of the other dihydrodiols tested to date (see Ref. 11), the 3,4-dihydrodiol of DMBA is by far the substrate most rapidly oxidized by dihydrodiol dehydrogenase. However, the enzyme appears to display absolute stereochemical preference for the 3*S*,4*S*-isomer. If this enantiomer contributes significantly to the formation of reactive diol-epoxides, then the dehydrogenase may play an important role in the detoxification of DMBA. Alternatively, if the diol-epoxides were to arise predominantly from the 3*R*,4*R*-dihydrodiol, then selective oxidation of the 3*S*,4*S*-dihydrodiol by the enzyme may effectively enrich the proportion of the 3*R*,4*R*-isomer available for diol-epoxide formation. Resolution of this issue must await complete elucidation of the stereochemical course of DMBA activation.

In summary, methylation of the parent hydrocarbon nucleus markedly influences the rate and stereochemical course of BA *trans*-3,4-dihydrodiol oxidation by dihydrodiol dehydrogenase and this may contribute to the differences in carcinogenicity observed in this series of compounds. Rapid, stereospecific oxidation of *trans*-dihydrodiol proximate carcinogens by this pathway may represent a previously unrecognized route of PAH metabolism. The overall contribution of dihydrodiol dehydrogenase to cellular PAH metabolism is currently under investigation in this laboratory.

[7] Absolute stereochemical assignment of the *trans*-3,4-dihydrodiols of 7-MBA, based on comparison of their CD spectra with those of the optically pure 3,4-dihydrodiol of BA, is valid because the 7-methyl group neither alters the conformation nor interacts with the asymmetrical centers of this dihydrodiol (23). The same argument applies to the assignment of the 3,4-dihydrodiol of 12-MBA based on comparison to CD spectra of optically pure DMBA diols (22).

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