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Stereospecificity of metabolism of benzo[a]pyrene (BP) to (±)*trans*-7,8-dihydroxy-7,8-dihydro-BP by rat liver nuclear enzymes*

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Recent studies [1–3] have shown that the potent carcinogen benzo[a]pyrene (BP) is metabolized by rat liver microsomes with high stereospecificity to (−)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BP 7,8-diol). Further microsomal metabolism results in the formation of diol-epoxides 1 and 2† which have been shown to form covalent adducts with nucleic acids [4–7]. Studies of nucleic acids derived from bovine and human bronchial explants incubated with benzo[a]pyrene showed that RNA contained adducts corresponding to the *trans* and *cis* addition of the 2 amino group of guanine to the 10 position of diol-epoxide 2 derived from (−)-BP 7,8-diol and corresponding to the *trans* addition of the 2 amino group of guanine to the 10 position of BP diol-epoxide 1 derived from the (+)-BP 7,8-diol [8]. Similar results were found for RNA derived from mouse skin treated with BP [9]. However, DNA from BP-treated bovine and human bronchial explants contained only one major nucleoside adduct corresponding to the *trans* addition of the 2 amino group of guanine to the 10 position of BP diol-epoxide 2 derived from the (−)-BP 7,8-diol [8].

Considerable evidence is now available which indicates that metabolic activation of BP can occur by nuclear mono-oxygenases as well as by microsomal systems [10–15]. Furthermore, the pattern of metabolites obtained from incubation of BP with the nuclear and microsomal systems appears to be qualitatively similar [13, 14]. The current investigation was undertaken to determine the stereospecificity of formation of BP 7,8-diol by rat liver nuclear enzymes, i.e. to ascertain if the predominant metabolite was identical to that observed in microsomal systems. The results of this study indicate that greater than 90 per cent of the 7,8-diol derivative of BP formed by rat liver nuclei is the (−)-isomer.

Eight male 50–100 g CD rats (Charles River Co.) were pretreated by i.p. injection with a solution of 3-methylcholanthrene (3-MC) (20 mg/kg) in corn oil. The animals were killed 24 hr later, their livers excised, and nuclei isolated as described previously [13, 14]. Nuclei were free of cytoplasmic contamination when examined by phase contrast microscopy. We have shown previously that the nuclei were uncontaminated when examined by electron microscopy as well [13].

[^{3}H]BP (5 Ci/mmole) was purified by alumina column chromatography and diluted to lower specific activity (2.5 Ci/mmol) with unlabeled BP that had been recrystallized from methanol. The nuclear incubation was performed in a total volume of 4.0 ml containing the following components: 200 μmoles Tris · Cl, pH 7.4; 200 μmoles EDTA; 18 μmoles glucose 6-phosphate (G-6-P); 2 μmoles NADPH; 6 μmoles $MgCl_2$; 12 units G-6-P dehydrogenase; nuclei equivalent to approximately 60 mg protein; and 1 mCi [^{3}H]BP (in 0.1 ml DMSO). After incubation for 30 min at 37°, the reaction was terminated by the addition of 4.0 ml acetone and 8.0 ml ethyl acetate. The metabolites were extracted into the organic phase and the aqueous phase was extracted two additional times with 4.0 and 2.0 ml, respectively, of ethyl acetate. The combined organic extracts were dried over anhydrous Na_2SO_4, and reduced to a residue under a stream of dry N_2.

The unmetabolized [^{3}H]BP, representing the bulk of the tritium labeled material, was removed from the ethyl acetate extract by elution from a 4 cm × 5 mm column of activity III basic alumina with petroleum ether–benzene (4:1, v/v). Oxygenated metabolites were recovered from the column by elution with 100% methanol [16]. The mixture of metabolites was again reduced to a residue under a stream of dry N_2 and the residue was redissolved in a minimal volume of methanol for analysis by high pressure liquid chromatography. High pressure liquid chromatography (h.p.l.c.) was performed with a Waters Associates instrument fitted with a 4 mm × 30 cm μBondapak C_{18} column. The metabolites were eluted at ambient temperature with a linear 60–80% methanol/water gradient at a flow rate of 1.0 ml/min and with a duration of 40 min; 0.2-ml fractions were collected and the radioactivity in the 7,8-diol region was pooled. This diol had a retention time of 20.4 min and its identity was confirmed by comparison with the retention times of authentic standards (a generous gift of Dr. D. M. Jerina of the NIAMDD). Resolution of BP 7,8-diol from other radioactive peaks was complete.

The [^{3}H]BP 7,8-diol isolated in this fashion was mixed with nonradioactive (±)-BP 7,8-diol (obtained from the NCI Chemical Repository through the NCI Carcinogenesis Research Program) and reacted with (−)-menthoxyacetyl chloride to give the di-(−)-menthoxyacetates according to the procedure of Yang *et al.* [17].

The diesters were separated by h.p.l.c. on a Waters Associates instrument with a μPorasil (4 mm × 30 cm) column at room temperature using 0.25% ethyl acetate (v/v) in methylene chloride as the eluting solvent at a flow rate of 2 ml/min. The stereoisomerism of the (−)-diester was confirmed by circular dichroism measurements. The (−)-diester yielded the corresponding (−)-BP 7,8-diol upon hydrolysis. The (−)-diester eluted with a retention time of 10.8 min, while the (+)-diester appeared at 12.8 min.

The radioactivity associated with the BP 7,8-diol, produced during the metabolism of BP by rat liver nuclei, co-chromatographed primarily with the (−)-isomer of the dimenthoxyacetate derivative of the synthetic (±)-BP 7,8-diol, the first peak shown in Fig. 1. The radioactivity in this peak corresponded to 92 per cent (−)-BP 7,8-diol with a small number of counts associated with the later eluting peak on h.p.l.c.; the maximum estimated proportion of radioactivity associated with (+)-BP 7,8-diols is thus 8 per cent of the total. A comparison with similar experiments using rat liver microsomal metabolism of BP is shown in Table 1. The production (−)-BP 7,8-diol is >90 per cent with both nuclear and microsomal enzyme systems. The stereospecificity of the BP-metabolizing enzymes of the two systems consequently must be regarded as substantially the same.

The similar stereospecificity of mono-oxygenase action in nuclei and microsomes reinforces previous studies [13, 14] in which we have shown that (a) reduced nuclear and microsomal hemoproteins undergo similar spectral shifts when carbon monoxide is added to systems derived from 3-MC-pretreated rats, (b) the spectrum of inducers of both nuclear and microsomal mono-oxygenases is identical, (c) the inhibition of both systems by a variety of substances is similar, (d) the BP metabolic products are qualitatively identical although the amounts of each differ after incuba-

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† Abbreviations: Diol-epoxide 1, 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10 tetrahydro-BP; diol-epoxide 2, 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10 tetrahydro-BP; BP 7,8-diol, (±)*trans*-7,8-dihydroxy-7,8-dihydro-BP; and DMSO, dimethylsulfoxide.

Table 1. Optical purity of benzo[a]pyrene 7,8-diol obtained on incubation of benzo[a]pyrene with 3-methylcolanthrene-induced rat liver-metabolizing systems

System	%(−)Enantiomer	Reference
Nuclei	92*	This work
Microsomes	97†	17
Microsomes	96*	3
Microsomes	92‡	3

* Value based on the amount of radioactivity associated with the respective diester separated by h.p.l.c.
† Value based on optical rotation measurements.
‡ Value based on circular dichroism measurements.

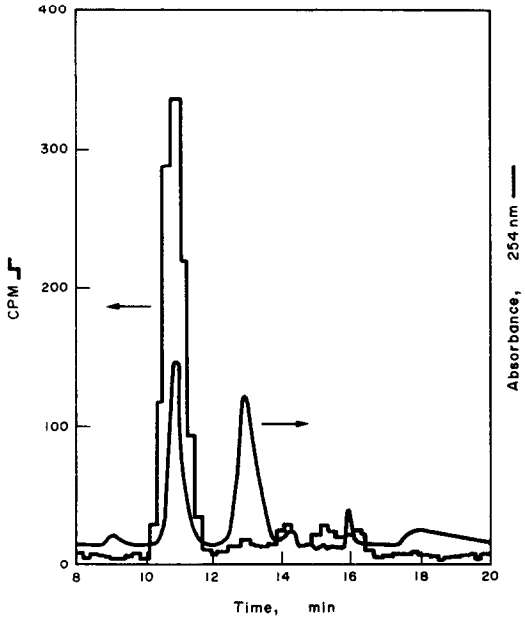

Fig. 1. High pressure liquid chromatographic profile of the di-(−)-menthoxyacetates of a mixture of synthetic (±)-BP 7,8-diol and BP 7,8-diol formed by incubation of [^{3}H]BP with 3-methylcholanthrene-induced rat liver nuclei. The early peak (10.8 min), the (−)-diester, corresponds to the (−)-BP 7,8-diol, and the later peak (12.8 min), the (+)-diester, corresponds to the (+)-BP 7,8-diol.

tion of nuclei or microsomes, and (e) antibody prepared against the purified microsomal cytochrome P-448 cross-reacts with the nuclear hemoprotein.*

Previous studies by Thakker *et al.* [3] have shown that liver microsomes from 3-MC-pretreated rats transform the (−)-isomer of BP 7,8-diol to predominantly diol-epoxide 1. It is of interest that both types of diol-epoxide are active mutagens in *S. typhimurium* and mammalian V79 cells [3, 18–21]. Furthermore, application of either diol-epoxide 1 or 2 to mouse skin elicits a most pronounced hyperplasiogenic effect [22]. The proximate or ultimate carcinogenic activity of the diol-epoxides appears to have been established by the experiments of Kapitulnik *et al.* [23], although skin tumor initiating activity after topical application of these BP derivatives is somewhat limited [24].

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