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DIFFERENCES BETWEEN PRODUCTS OF BINDING OF 7,12-DIMETHYLBENZ[a]ANTHRACENE TO DNA IN MOUSE SKIN AND IN A RAT LIVER MICROSOMAL SYSTEM

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SUMMARY

Hydrocarbon-deoxyribonucleoside products from the DNA of mouse skin exposed in vivo to 7,12-dimethylbenz[a]anthracene are chromatographically the same as the products formed in mouse embryo cell cultures. These products, which are known to arise through the generation of a diol-epoxide in the 1,2,3,4-ring of the hydrocarbon, are chromatographically separable from products that result from reaction of the K-region oxide of this hydrocarbon with DNA. However, when 7,12-dimethylbenz[a]anthracene is bound to DNA in the presence of a microsomal system analogous to those used in various carcinogen detection systems, the hydrocarbon-deoxyribonucleoside products co-chromatograph with the K-region oxide products. Differences in the profiles of metabolites formed in mouse embryo cell cultures and rat liver microsomal systems are consistent with the differences between the DNA-bound products in these two systems.

INTRODUCTION

Microsomal preparations, cell homogenates, or fractions of cell homogenates are frequently used in vitro to mimic the metabolic activation of carcinogens which occurs in their respective target tissues in vivo (e.g. 1-5). The tacit assumption of similar metabolic pathways in these subcellular fractions and in target tissues is difficult to test directly since metabolites cannot be satisfactorily monitored in specific tissues in vivo. The chemically reactive metabolites formed in vivo can be measured indirectly, however, by determining the reactive metabolites which bind with DNA in the target tissue.

It has recently been shown that binding of the potent hydrocarbon carcinogen, 7,12-dimethylbenz[a]anthracene (DMBA)[1] to DNA in rodent embryo cell cultures involves the generation of a diol-epoxide in the 1,2,3,4-ring (6-8). In this paper, it is reported that DMBA binds to DNA in target tissue (mouse skin)

[1]Abbreviations used are: DMBA, 7,12-dimethylbenz[a]anthracene; DMBA 5,6-oxide, 7,12-dimethylbenz[a]anthracene 5,6-oxide; TCPO, 1,1,1-trichloropropylene oxide; HPLC, high pressure liquid chromatography.

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through the same mechanism, whilst its microsome-catalyzed binding to DNA *in vitro* occurs through a different reactive intermediate, namely DMBA 5,6-oxide[1], the K-region epoxide.

MATERIALS AND METHODS

[^{3}H]DMBA (specific radioactivity, 12 Ci/mMole) was routinely purified by thin layer chromatography (Eastman chromatogram sheet 13181 silica gel developed with n-hexane) prior to use and diluted to an appropriate specific radioactivity where necessary. DMBA 5,6-oxide was prepared according to Dansette and Jerina (9) and was characterized by mass spectrometry. 7-Hydroxymethyl-12-methylbenz-[a]anthracene, 12-hydroxymethyl-7-methylbenz[a]anthracene, and *cis*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene were prepared by published procedures (10,11).

DMBA-DNA Products. [^{3}H]DMBA (0.6 mCi/mouse) in benzene (0.1 ml) was applied to the shaved backs of 4 female NIH Swiss mice. After 24 hours the mice were killed and DNA was isolated from the excised skins by the phenol method and purified by centrifugation in CsCl density gradients, all as previously described (12).

DNA was isolated from primary mouse embryo cell cultures which had been treated with [^{3}H]DMBA as described by Moschel *et al.* (6).

The microsome catalyzed binding of DMBA to DNA followed the procedures of King *et al.* (13). Male Sprague-Dawley rats weighing approximately 200 g were injected with Aroclor 1254 (i.p. 500 mg/kg) and killed on the fifth day thereafter. Liver microsomes were then prepared (13) and incubated with co-factors, [^{3}H]DMBA (specific radioactivity 3.1 Ci/mMole; 80 μM), calf thymus DNA (3 mg/ml), with or without 1,1,1-trichloropropylene oxide (TCPO)[1] (80 μM) at 37° for two hours (13). DNA was then recovered using the phenol procedures, followed by extensive washing with ethyl acetate, benzene and ether until no organic solvent soluble radioactivity remained (13). Control incubations which contained no microsomes or boiled microsomes accompanied each microsomal incubation.

A solution of calf thymus DNA (1 mg/ml) was treated with DMBA 5,6-oxide, and the modified DNA was recovered according to the procedures of Blobstein *et al.* (14).

Analysis of Modified DNA Samples. Purified DNA preparations were enzymically degraded to mixtures of deoxyribonucleosides (12) and examined by chromatography on Sephadex LH20 eluted with a methanol/water gradient (15). The UV absorbance at 254 nm in chromatographic fractions was monitored to detect the presence of DNA-DMBA 5,6-oxide products, and radioactivity in aliquots (1 ml) of each fraction (4.5 ml) was measured to locate the various [^{3}H]DMBA-DNA products. Further chromatography of the DMBA 5,6-oxide-nucleoside products was by high pressure liquid chromatography (HPLC)[1] (Spectra Physics) on a Dupont Zorbax ODS column (0.25 m x 4.6 mm) using a concave gradient (No. 4) from 35% to 95% methanol in water at a flow rate of 1.7 ml/minute for 60 minutes.

Metabolism Studies. Culture medium (250 ml) from the primary embryo cell cultures, above, was extracted with ethyl acetate (3 x 30 ml). A microsomal incubation with [^{3}H]DMBA in the absence of DNA was quenched by the addition of NaCl (1 g) and the mixture (8 ml) was similarly extracted with ethyl acetate (3 x 3 ml). After drying the extracts over $MgSO_4$, aliquots were examined by HPLC using a Spectra Physics Modular HPLC equipped with Model 740B pumps. Metabolites were separated on a Whatmann Partisil PXS 10/25 ODS-2 column (0.25 m x 4.6 mm) using a concave gradient (No. 4) of 35% to 80% acetonitrile in water for 55 minutes at a flow rate of 1.4 ml/minute. Added UV absorbing markers were detected by continuously monitoring the absorbance of the column effluent at 254 nm. Radioactive metabolites were detected by liquid scintillation counting of each fraction (1.4 ml).

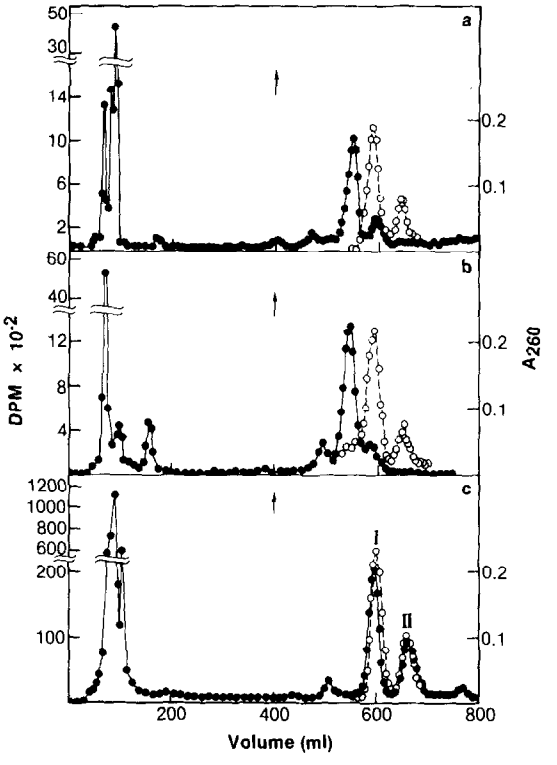

Figure 1: Comparison of Sephadex LH20 chromatography of DMBA 5,6-oxide-nucleoside products (O——O) formed by enzymic digestion of calf thymus DNA which had been reacted with DMBA 5,6-oxide in vitro with enzymically digested DMBA-DNA products from (a) mouse skin from animals treated 24 hours with [^{3}H]DMBA; (b) mouse embryo cell cultures treated 24 hours with [^{3}H]DMBA; and (c) calf thymus DNA treated with [^{3}H]DMBA for 2 hours in the presence of Aroclor-stimulated rat microsomes. ●——●, DPM; O——O, absorbance at 260 nm; ↑, position for elution of added 4-(p-nitrobenzyl)pyridine marker.

RESULTS AND DISCUSSION

Since the mechanism of metabolic activation of DMBA in rodent embryo cell cultures is reasonably well understood (6-8), the products of binding of this carcinogen to DNA in mouse skin in vivo and in a rat liver microsomal system in vitro were both compared with the products of binding which arise in primary mouse embryo cell cultures (Fig. 1). In order to facilitate comparison UV absorbing markers from an enzyme digestion of DNA that had been reacted with DMBA 5,6-oxide in vitro were included in each column chromatogram. The nature of the radioactive products which elute in the first 120 ml of eluant is not

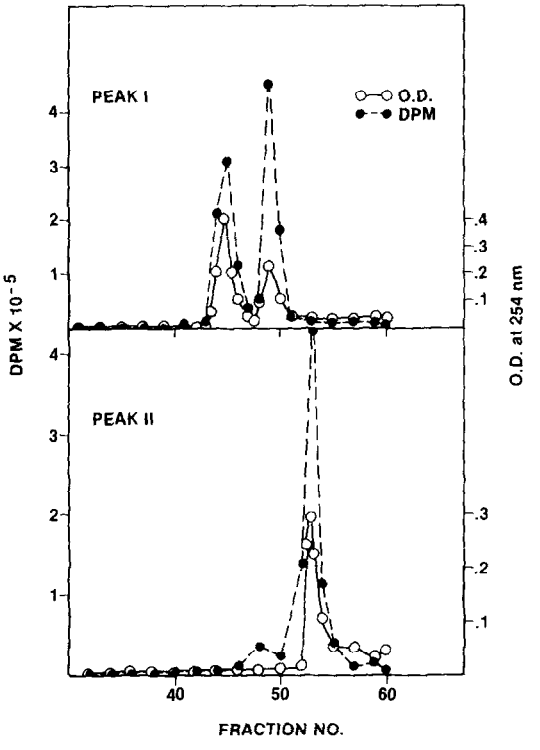

Figure 2; High pressure liquid chromatography of peaks I and II (see Figure 1c) of [^{3}H]DMBA-nucleoside products (●——●) and DMBA 5,6-oxide-nucleoside products (O——O).

clear, but hydrocarbon-nucleoside adducts usually elute after more than 400 ml of eluant has been collected as evidenced in Figure 1 by the radioactive and UV absorbing peaks in this region.

It is clear that the radioactive DMBA-nucleoside products from mouse skin (Fig. 1a) and from mouse embryo cell cultures (Fig. 1b) behave identically in the Sephadex LH20 chromatography system, and that the mechanism of binding of DMBA to DNA in mouse skin presumably involves, therefore, a 1,2,3,4-ring diol-epoxide, as does the binding process in the embryo cells (6-8). In concert with this, neither the skin nor the embryo cell products co-chromatograph with the K-region epoxide products (Figs. 1a and 1b). However, when DMBA is activated for binding to DNA in an Aroclor-induced rat liver microsomal system, the hydrocarbon-nucleoside products elute from this chromatographic system almost in

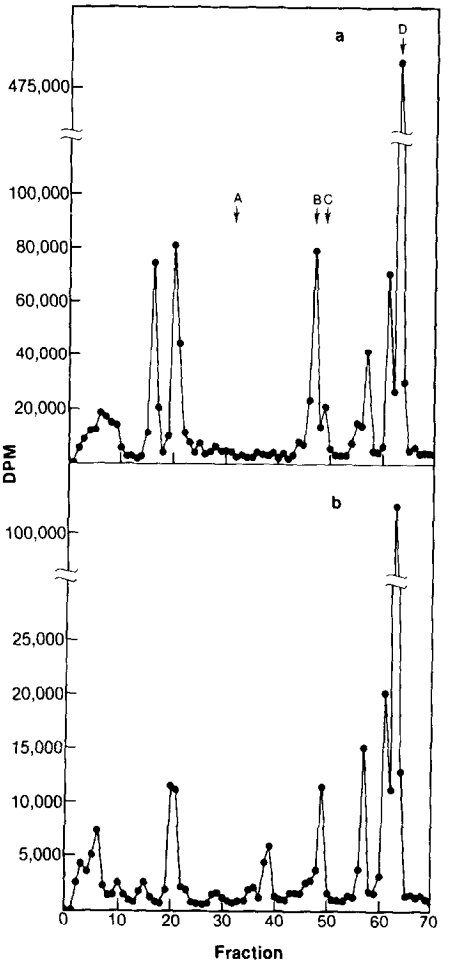

Figure 3: High pressure liquid chromatography of ethyl acetate soluble DMBA metabolites from (a) Aroclor-stimulated rat liver microsomes treated with [^{3}H]DMBA for 30 minutes and (b) mouse embryo cell cultures treated with [^{3}H]DMBA for 24 hours. The arrows indicate positions of elution of added UV absorbing markers: A, cis-5,6-diol-DMBA; B, 7-hydroxymethyl-12-methyl-benz[a]anthracene; C, 12-hydroxy-methyl-7-methylbenz[a]anthracene; and D, DMBA.

coincidence with the K-region epoxide marker products (Fig. 1c). When peaks I and II (Fig. 1c) were recovered and separately chromatographed on an HPLC system (Fig. 2), it became clear that the microsomally-bound products and the K-region oxide products were in fact the same. The slight discrepancy in the position of elution of the radioactive and UV absorbing components of peak I in

the LH20 system (Fig. 1c) is due to the fact that this peak is composed of at least two components which are present in different proportions in the microsomally-bound products and the K-region oxide products.

These experiments clearly show that Aroclor-stimulated rat liver microsomes do not mimic the metabolic activation of DMBA which occurs in the target tissue, mouse skin, or in mouse embryo cell cultures. Another illustration of this difference is that the epoxide hydrase inhibitor, TCPO, inhibits the binding of DMBA to DNA in mouse embryo cell cultures as would be expected (8), but that, in concert with the general finding in other laboratories, TCPO (80 μM) slightly enhances the binding of DMBA to DNA in the microsomal system (i.e., binding was 95 and 70 μmoles hydrocarbon/mole DNA phosphorus in the presence and absence of TCPO respectively).

The DMBA metabolites formed in the embryo cell culture and in the microsomal system have also been compared (Fig. 3). In the absence of all of the appropriate marker metabolites, a detailed interpretation of each of these metabolite profiles is not possible. It is evident, however, that in both systems a metabolite with a retention time of 20 mins is a major component. Sims has previously shown that the major DMBA metabolite in mouse embryo cell cultures is the 8,9-dihydrodiol (16) and the UV absorption spectrum of the metabolite eluted at 20 mins is consistent with that assigned previously to this metabolite (17). In the microsomal metabolism (Fig. 3a), the metabolite (retention time 16 mins), which is absent or present in low amounts in the cell culture system, is probably the trans-5,6-dihydrodiol. This follows because its UV absorption spectrum is identical to that reported for the trans-diol by Boyland and Sims (18) and it was clearly separated from the cis-isomer in the chromatographic system. The trans-5,6-dihydrodiol (19) would be expected to be present since the 5,6-oxide is generated in this system (Figs. 1 and 2).

These findings demonstrate that the Aroclor-stimulated microsomal system does not mimic the metabolic activation of DMBA which occurs in a target tissue for carcinogenesis, mouse skin. Similar conclusions for 7-methylbenz[a]anthra-

cene (19) and benzo[a]pyrene (13) with respect to 3-methylcholanthrene induced rat liver microsomes have been reported. Whilst it would be unwise to extrapolate these conclusions to other classes of carcinogens and to other in vitro metabolic activation systems, these findings emphasize that the appropriateness of various in vitro metabolic activation systems should be experimentally established, not simply assumed.

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