THE NATURE OF BENZO(A)PYRENE-DNA ADDUCTS FORMED IN HAMSTER EMBRYO CELLS DEPENDS ON THE LENGTH OF TIME OF EXPOSURE TO BENZO(A)PYRENE

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SUMMARY: Most of the benzo(a)pyrene-DNA adducts found in hamster embryo cells after 4 or 6 hr of benzo(a)pyrene treatment resulted from reaction with (±)7α,8β-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)-pyrene (the syn-isomer) whereas most of the adducts found at 24 or 72 hr resulted from reaction with (±)7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (the anti-isomer).

INTRODUCTION

To identify the minute amounts of hydrocarbon-DNA interaction products formed in vivo and in cell culture (1-4), a chromatographic method was developed to isolate radioactive hydrocarbon-DNA adducts from cells and to compare these with adducts formed by reactive hydrocarbon derivatives in solution (5, 6). The use of this technique demonstrated that the benzo(a)pyrene (BP)[1]-DNA adducts formed in mouse or hamster embryo cell cultures were not the same as those formed when the K-region epoxide of BP reacted with DNA (7, 8), but were identical to those formed by reaction of BP-diol-epoxide with DNA (8). Binding of BP to DNA and RNA in other cells in culture and in animal tissue also involves BP-diol-epoxide (9-13). It was proposed that syn- and anti-isomers of this diol-epoxide might be formed (14), and both were synthesized and their DNA adducts characterized (15-17). King et al. (13) separated the BP-DNA adducts of the syn- and anti-isomers by adding sodium borate to the eluant of LH20 columns and suggested that the adducts formed in vivo resulted mainly from the anti-isomer.

[1]Abbreviations used are: BP, benzo(a)pyrene; syn-diol-epoxide, (±)7α,8β-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene; anti-diol-epoxide, (±)7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene.

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However, Shinohara and Cerutti (18) have shown that DNA adducts of both isomers are formed in cultures of mouse embryo fibroblasts and BHK21/C13 cells and that these are repaired slowly. We have examined the BP-DNA adducts formed in hamster embryo cells treated for various lengths of time with BP to determine if the ratio of adducts formed from the two isomers is constant.

MATERIALS AND METHODS

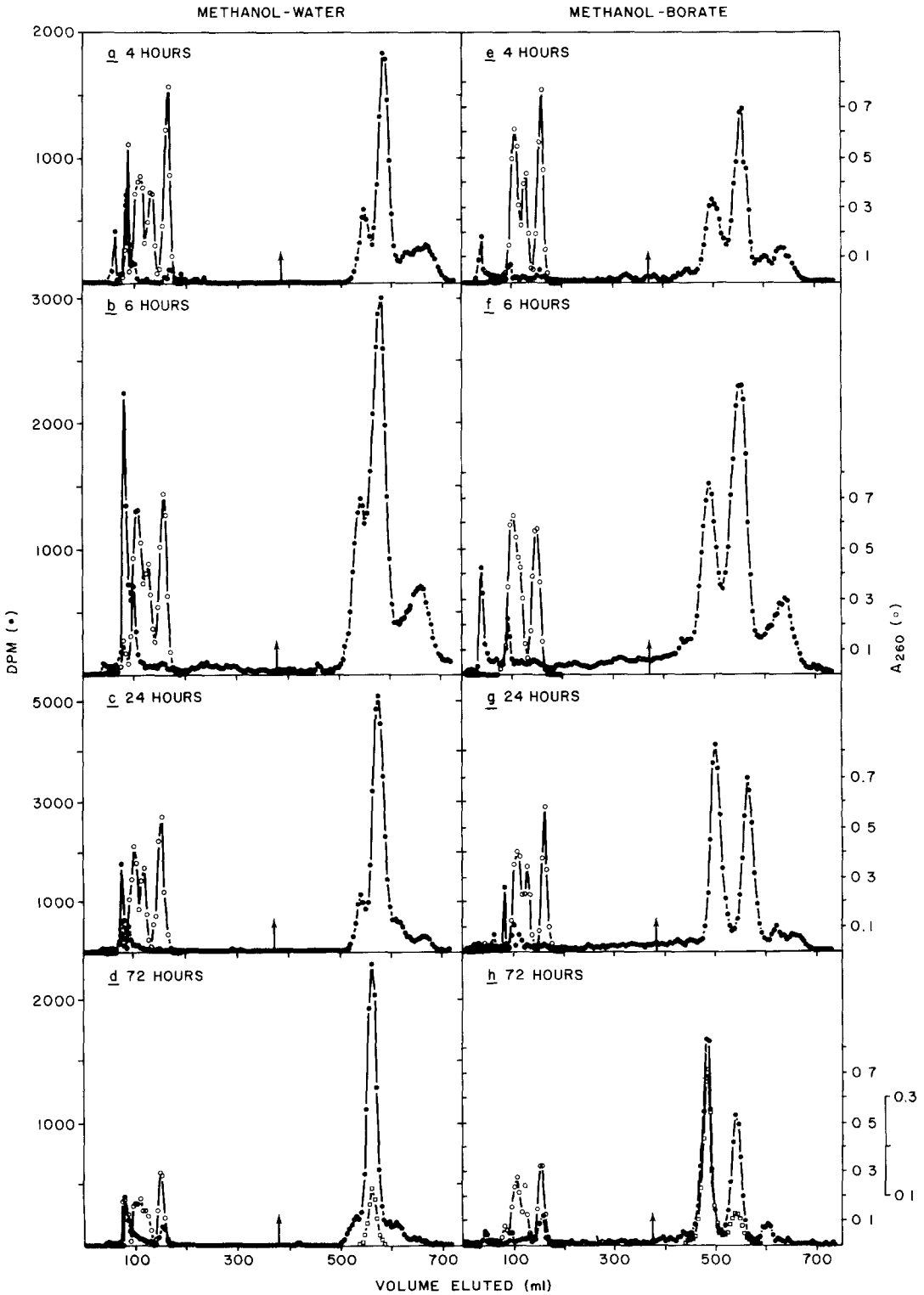
Binding of [^{3}H]BP to DNA: Second passage Syrian hamster embryo cells growing in monolayer culture were treated with [G-^{3}H]BP (Amersham-Searle; S.A. 8.3 Ci/mmole; 0.5 nmole/ml medium) for the lengths of time specified and DNA was isolated by a modification (19) of the Kirby phenol procedure (20). The total amount of [^{3}H]BP metabolized at each time point was determined (19).

Analysis of [^{3}H]BP-DNA Adducts: DNA samples dissolved in 0.01 M Tris, 0.01 M $MgCl_2$, pH 7.0, were divided in half and degraded to deoxyribonucleosides with the enzymes DNAse I (bovine pancreas), phosphodiesterase (C. atrox venom) and alkaline phosphatase (E. coli type III) (Sigma Chemical Co). DNA digests were chromatographed on 90 x 1.5 cm columns packed with Sephadex LH20 to a height of 80 cm eluted with a linear gradient composed of either methanol:water (3:7) to methanol (5,7) or methanol:0.05 M sodium borate pH 8.7 (3:7) to methanol (13). In the latter case, the column was prerun with the methanol:borate mixture for 2 to 3 hr prior to application of the sample. Fractions (150) of approximately 5 ml were collected, the radioactivity in 1.0 ml samples of each was determined by liquid scintillation counting and the UV-absorbance of each fraction was measured.

Preparation of BP-diol-epoxide-DNA Products: Syn-(#152) and anti-(#137) diol-epoxides (Chemical Repository, N.C.I.) were reacted with deproteinized (21) calf thymus DNA for 18 hr at 37° as described by Osborne (15). After extraction with ethyl acetate (16) the DNA was precipitated twice, washed with ethanol:water (7:3) and ethanol, and dried under N_2. After enzyme digestion, the DNA samples were chromatographed on LH20 columns eluted with methanol:water gradients. Each sample contained one major UV-absorbing peak that eluted at 570 ml. The UV-absorption spectra of both peaks were identical to those of BP-diol-epoxide-nucleoside and -nucleotide adducts (16, 17). When chromatographed on silica gel thin-layer plates developed in chloroform:methanol (4:1) (22), the DNA adducts from both samples remained at the origin, while ethyl acetate-extractable derivatives from the above reaction had R_f's: syn, 0.6, 0.2, 0.1, and anti, 0.6, 0.3.

RESULTS

The methanol-water gradient elution profiles of Sephadex LH20 columns of BP-DNA adducts formed in hamster embryo cells after BP treatment for various lengths of time are shown in Fig. 1a-d. The 4 hr DNA digest contains two sharp peaks and a smaller broad peak of BP-deoxyribonucleoside


METHANOL-WATER
METHANOL-BORATE
a 4 HOURS
e 4 HOURS
b 6 HOURS
f 6 HOURS
c 24 HOURS
g 24 HOURS
d 72 HOURS
h 72 HOURS
DPM (•)
A260 (○)
VOLUME ELUTED (ml)

products (Fig. 1a; elution volume 500 ml to 700 ml). After 6 hr of BP treatment, there was no longer a distinct first peak but there was a large shoulder on the second peak (Fig. 1b). After 24 hr or 72 hr (Fig. 1c and d), only a small shoulder was present on the second major peak. These results suggested that there was more than one major product at early times after BP treatment. We, therefore, examined digests of the same samples of DNA on columns eluted with methanol-borate gradients (Fig. 1e-h). The elution profile of the 4 hr DNA digest contained two major peaks: the first, the smaller of the two, eluted in the same region of the gradient as the DNA adducts isolated from DNA reacted with the anti-diol-epoxide; the second peak eluted in the same region as the adducts isolated from DNA reacted with the syn-diol-epoxide. When UV-absorbing markers of the syn- and anti-diol-epoxide-DNA adducts were chromatographed with the 72 hr DNA sample (Fig. 1h) or the products (Fig. 1b) from the 6 hr DNA sample (not shown), the radioactive peaks cochromatographed with the two markers.

The two radioactive peaks isolated from each of the methanol-borate columns were further characterized by thin-layer chromatography. Both cochromatographed with the UV-absorbing markers of the anti- and syn-diol-epoxide-deoxyribonucleoside products on silica gel G thin-layer plates developed in acetone (R_f 0.0) and in ethyl acetate:methanol:water:formic acid (100:25:20:1) (R_f 0.4).

Fig. 1 LH20 column elution profiles of enzyme digests of DNA from hamster embryo cells treated with [^{3}H]BP for the length of time specified. Columns on the left were eluted with gradients of 500 ml methanol:water (3:7) to 500 ml methanol. Columns on the right were eluted with gradients of 500 ml methanol:0.05 M sodium borate (3:7) to 500 ml methanol. The same amounts of DNA were applied to both columns for each time point: 4 hr, 1.1 mg DNA (5.4 pmole BP/mg DNA); 6 hr, 1.2 mg DNA (10.5 pmole BP/mg DNA); 24 hr, 0.8 mg DNA (17.9 pmole BP/mg DNA); 72 hr, 0.6 mg DNA (7.7 pmole BP/mg DNA). The percentage of [^{3}H]BP metabolized by the cultures was: 4 hr, 23%; 6 hr, 40%; 24 hr, 94%; 72 hr, 99%. ↑, p-nitrobenzylpyridine. • DPM/1 ml sample of each fraction. o A_{260} of each fraction. □ A_{246} of fractions containing diol-epoxide-DNA adduct markers.

The borate elution profiles showed that after 4 and 6 hr of BP treatment the first peak (anti) was smaller than the second (syn) (Fig. 1e, f). However, after 24 hr of treatment, the first peak contained slightly more BP-DNA products than the second (Fig. 1g). After 72 hr, the first peak, which cochromatographed with a marker of anti-diol-epoxide-DNA products, was 1.4 times as large as the second peak, which cochromatographed with a marker of syn-diol-epoxide-DNA products (Fig. 1h).

DISCUSSION

These results show that the DNA-bound products formed in hamster embryo cells at early times after treatment with BP are formed mainly through reaction with the syn-diol-epoxide, while at later times the products are mainly those formed by reaction with the anti-diol-epoxide. The explanation for this difference is not known. Shinohara and Cerutti (18) found that in mouse embryo fibroblasts the syn-diol-epoxide product was excised slightly faster than the anti, but it seems unlikely that the slow rate of excision they observed could account for the dramatic changes in the amounts of syn- and anti-diol-epoxide products in hamster embryo cells at 6 and 24 hr. The proportion of each isomer being formed may change with time or the anti-isomer, which is more stable than the syn (23), may continue to bind to DNA over a longer period of time and ultimately reach a higher binding level than the syn-isomer. Alternatively, there may be DNA adducts formed at early times that are rapidly excised and not found later. Clearly both diol-epoxide isomers play an important role in the binding of BP to DNA in hamster embryo cells and the nature, as well as the amount of BP-DNA adducts found, is dependent upon the length of time of exposure to the hydrocarbon.

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