METABOLIC ACTIVATION OF THE CARCINOGEN 7,12-DIMETHYLBENZ[a]ANTHRACENE FOR DNA BINDING

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SUMMARY

Isolation of hydrocarbon-deoxyribonucleoside products from the DNA of mouse embryo cells exposed to 7,12-dimethylbenz[a]anthracene permits both fluorescence excitation and emission spectra to be recorded. Comparison of these spectra with those of various model compounds indicates that 7,12-dimethylbenz[a]anthracene, one of the most potent of the hydrocarbon carcinogens, is metabolically activated for DNA binding through the generation of a diol-oxide in the 1,2,3,4-ring.

INTRODUCTION

Recent progress in understanding the mechanisms of action of the polycyclic aromatic hydrocarbons has stemmed from studies of the metabolically-mediated binding of these carcinogens to DNA (1,2). This has led to the identification of 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene 9,10-oxide as the benzo[a]pyrene metabolite involved in DNA binding (3), and to the suggestion that the metabolic formation of diol-oxides in the "bay regions" (4) of hydrocarbons might be a general mechanism of metabolic activation for these carcinogens (5).

In this paper, the fluorescence excitation and emission spectra of the products formed between 7,12-dimethylbenz[a]anthracene (DMBA)[1] and DNA of mouse embryo cell cultures are reported. These indicate that DMBA is metabolically activated through the generation of a diol-oxide in the 1,2,3,4-ring. This is consistent with the "bay region" generalization noted above.

MATERIALS AND METHODS

[G-^{3}H] DMBA (Amersham/Searle Corporation) and 9,10-dimethylanthracene (Sigma Chemical Co.) were obtained commercially. 8,9,10,11-Tetrahydro-DMBA

[1]Abbreviation used is DMBA, 7,12-dimethylbenz[a]anthracene.

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(6) was provided by Dr. W. Lijinsky, Frederick Cancer Research Center, trans-3,4-dihydro-3,4-dihydroxybenz[a]anthracene by Dr. D.M. Jerina, National Institute of Arthritis, Metabolism and Digestive Diseases, and cis-5,6-dihydro-5,6-dihydroxy-DMBA was prepared according to Cook and Schoental (7).

DMBA-DNA Products. Primary cultures of Swiss mouse embryo cells were grown in Eagle's MEM supplemented with 10% fetal bovine serum either in plastic flasks (150 cm^2) or in roller bottles (450 cm^2) (8). Confluent monolayers were treated with [^{3}H]DMBA (0.3 µg/ml; specific radioactivity 0.3 Ci/mmole) for 24 hours. The hydrocarbon was added in dimethylsufoxide solution so that the final concentration of this solvent in the medium was 0.5%. Cells were harvested by trypsinization, and DNA was isolated by phenol extraction and purified by ribonuclease treatment and the methoxyethanol-phosphate procedure, all as previously described (9). DNA was then degraded enzymically to deoxyribonucleosides (9). The radioactive DMBA-DNA products were isolated by chromatography on Sephadex LH-20 eluted with a gradient from 500 ml methanol/water (3:7) to 500 ml methanol (10). Aliquots were taken from each fraction (5 ml) for determination of radioactivity by liquid scintillation counting.

Fluorescence Studies. Fractions containing radioactive DMBA-DNA products were pooled, evaporated to dryness, and re-dissolved in ethylene glycol/water (1:1, 0.2 ml). Technical fluorescence excitation and emission spectra (i.e. uncorrected for lamp response and photomultiplier sensitivity) were measured at room temperature in quartz tubes using a Perkin Elmer MPF3 fluorescence spectrophotometer and phosphorescence accessory. Spectra were also recorded after further purification of the products on a column of µBondapak C-18 (Waters Associates, Milford, Mass.) (0.67 x 30 cm) eluted with a gradient from 25 ml methanol/water (3:7) to 25 ml methanol. The products were again detected by their radioactivity, appropriate fractions (1 ml) were pooled, evaporated, and the products were re-dissolved as above. The fluorescence spectra of the various model compounds used were measured under the same conditions used for the DMBA-DNA products.

RESULTS AND DISCUSSION

Previous studies have shown that hamster embryo cell DNA-DMBA adducts are highly photosensitive compounds as are 9,10-dimethylanthracene and DMBA itself (11). DMBA derivatives saturated in the 8,9,10,11-ring or at the 5,6-double bond are not particularly photosensitive and, therefore, it was suggested that, in the binding reaction with DNA, aromaticity in the dimethylanthracene system must be retained. Metabolic activation must occur then, either in the 1,2,3,4-ring or possibly through an intermediate which would leave the whole DMBA aromatic system intact (11). This fluorescence study was undertaken in order to substantiate these general conclusions and to define more precisely the DMBA metabolite involved in binding to DNA. Mouse embryo cells were preferred over hamster cells for this study because higher levels of binding to DNA are obtained.

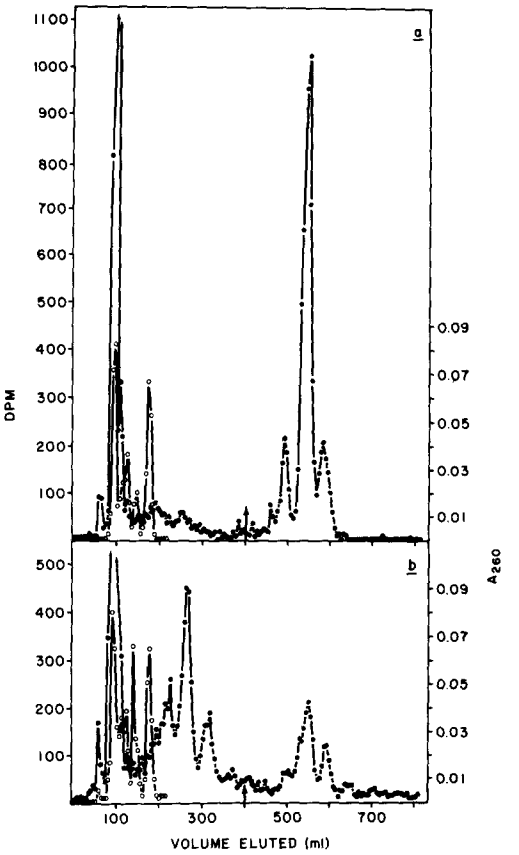

Figure 1. Chromatography on Sephadex LH-20 of DMBA-DNA products after enzymic digestion to deoxyribonucleosides. Mouse embryo cells were treated with [^{3}H]DMBA (0.07 µg/ml; specific radioactivity, 12 Ci/mmol) for 24 hours, and DNA with 18.7 µmoles hydrocarbon bound per mole of DNA phosphorus was isolated, enzymically degraded to nucleosides and chromatographed as described under Material and Methods. The enzyme digest (equivalent to 0.1 µg DNA) was kept in the dark (a) or exposed to light from two 4-watt fluorescent tubes (Microscope Light, A.H. Thomas, Philadelphia, Pa.) at a distance of 5 cm for 8 hours (b). ●——●, dpm; o——o, absorbance at 260 nm; ↑, position for elution of added 4-(p-nitrobenzyl)pyridine marker.

Figure 1 illustrates that the chromatographic elution profile of mouse embryo cell DNA-DMBA products (after enzymic digestion to deoxyribonucleosides) is similar to that of the hamster DNA products (11). Furthermore, the products eluted after 450 ml eluant has passed through the column are similarly photosensitive as demonstrated by the dramatic change in elution profile when the nucleoside mixture is irradiated with visible light prior to chromatography (Fig. 1b). The radioactive products eluted in 80-120 ml eluant have not been

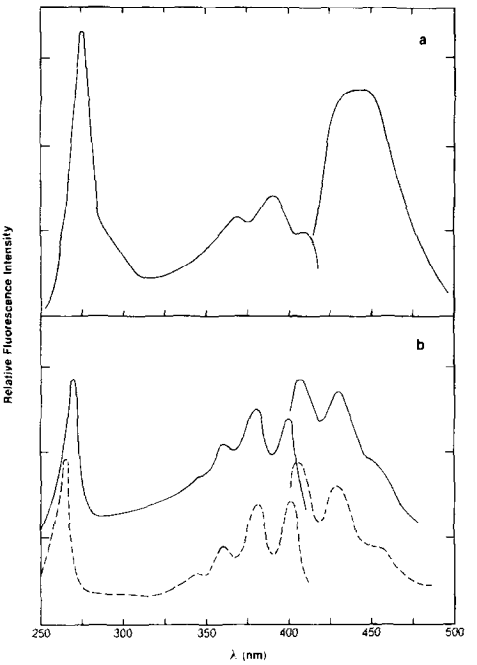

Figure 2. Technical fluorescence excitation and emission spectra at room temperature for (a) DMBA-DNA deoxyribonucleoside products isolated from mouse embryo cell DNA containing 70 μmoles hydrocarbon per mole DNA phosphorus (obtained as described under Materials and Methods); (b) DMBA-DNA deoxyribonucleoside products after heating at 60° for 9 minutes in ethylene glycol/1.4N HCl (1:1, v/v) (———) and 9,10-dimethylanthracene (----) measured under the same acidic conditions. For the DMBA-DNA products (a), the excitation spectrum was determined with emission at 440nm, and the emission spectrum with excitation at 275nm. After acid hydrolysis (b), the corresponding settings used were 430nm and 270nm, and for dimethylanthracene (b) 430 and 264nm.

studied in any detail though it has been established that they represent only 5-10% of the total binding if the carcinogen is incubated with the cells for 48 hours rather than for 24 hours as used here.

Mouse embryo cells were grown in sufficient quantity to permit the isolation of 4 mg DNA. This was digested enzymically and chromatographed on the Sephadex LH-20 system yielding an elution profile essentially as illustrated in Figure 1a. Fractions corresponding to the radioactive products (475-615 ml, Fig. 1a) were pooled and their fluorescence was examined. After further purification on a μBondapak column, the fluorescence excitation and emission spectra were recorded again (Fig. 2a). This latter chromatographic step reduced the fluorescent background and also demonstrated that the

TABLE I

FLUORESCENCE EXCITATION AND EMISSION DATA[a]

Compound	Emission (λ, nm)[b,c]	Excitation (λ, nm)[b,d]
DMBA	407,428,450(sh)[e]	291,302,352,(sh),366,384(sh)
8,9,10,11-Tetrahydro-DMBA	376(sh),387,405(sh)	271,308(sh),315
cis-5,6-Dihydro-5,6-dihydroxy-DMBA	355(sh),367	274,308
trans-3,4-Dihydro-3,4-dihydroxybenz[a]-anthracene	415,438,465(sh)	267,352(sh),367,387,409

[a]See Material and Methods for conditions. [b]Relative maxima are underlined. [c]Fluorescence emission spectra were measured with excitation at the longest wavelength excitation maximum. [d]Excitation spectra were measured with emission at the emission maximum. [e]Shoulder is indicated by (sh).

fluorescence was eluted with the radioactive products in a second chromatographic system. In both systems, fractions on either side of the radioactive peaks were pooled, evaporated, and their fluorescence was examined. These did not exhibit the fluorescence associated with the radioactive products.

None of the fluorescence spectra of the various model compounds examined (Table 1) was exactly like those of the products. 8,9,10,11-Tetrahydro-DMBA, a model for binding through a diol-oxide in the 8,9,10,11-ring, exhibited a long wavelength excitation peak at 315nm and an emission maximum at 387nm, both at considerably shorter wavelength than the products. A model for binding through a K-region oxide, cis-5,6-dihydro-5,6-dihydroxy-DMBA, similarly exhibited an excitation maximum (308nm) and emission maximum (367nm) at much shorter wavelength than the products. DMBA itself was notably different in short wavelength excitation maximum (302nm), and the longer wavelength maxima,

though closer to the products than the other models, were still at much shorter wavelength (352, 366, 384nm). The excitation spectrum of the 3,4-dihydrodiol of benz[a]anthracene (12) was quite close to that of the products. However, this model does not contain methyl groups in the 7 and 12 positions and comparison of the fluorescence of anthracene and benz[a]anthracene with that of 9,10-dimethylanthracene and DMBA suggests that the methyl groups shift the fluorescence excitation about 20nm towards longer wavelength. Although the excitation spectra of 9,10-dimethylanthracene and the products are similar in overall shape, they are not superimposable (Fig. 2b). Since this model compound does not contain a bound nucleoside residue, an exact spectral match is not expected. However, if binding did occur through a diol-oxide, the hydrocarbon-nucleoside bond should be susceptible to acid hydrolysis as reported for the products of reaction of benzo[a]pyrene diol-oxide with poly-guanylic acid (13). A dramatic change in the fluorescence of the DMBA products was observed after acid hydrolysis. The emission and excitation spectra of the hydrolysis products (presumably now containing a DMBA tetrol) were almost indistinguishable from those of 9,10-dimethylanthracene (Fig. 2b).

These findings on the photosensitivity and fluorescence properties of DMBA-DNA products clearly show that the bound hydrocarbon is fully saturated in the 1,2,3,4-ring. The most obvious interpretation is that reaction with DNA occurs through the generation of a diol-oxide in the 1,2,3,4-ring. However, these findings do not indicate whether a 1,2-diol 3,4-oxide or the isomeric "bay region diol-oxide" (3,4-diol 1,2-oxide) is the metabolically-generated reactive intermediate.

Earlier fluorescence studies in this area (14-16) have dealt only with the emission spectra of the modified DNA itself. The present studies extend the usefulness of this approach by demonstrating that, with suitable procedures for the isolation of products, excitation spectra can also be obtained. Further more, in the absence of the exact model required for spectral comparison, acid

hydrolysis of the hydrocarbon-nucleoside bond will permit comparisons to be made with models based only on the hydrocarbon residue's structure.

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