METABOLIC ACTIVATION OF POLYCYCLIC HYDROCARBONS

Fluorescence spectral evidence is consistent with metabolism at the 1,2- and 3,4-double bonds of 7-methylbenz[a]anthracene+

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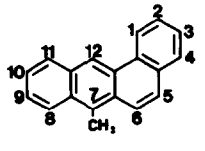
Received 1 February 1977

1. Introduction

When photon-counting spectrophotofluorimetry was first applied to studies on the in vitro metabolic activation of the carcinogenic polycyclic hydrocarbons [1–3], the initial studies showed that the K-region epoxide of 7-methylbenz[a]anthracene did not appear to be the species that reacted with nucleic acid in cells treated with the parent hydrocarbon [1]. This technique was later used to confirm that the metabolic activation of benzo[a]pyrene involved a vicinal diol-epoxide formed on the adjacent 7,8- and 9,10-double bonds [3]. We now wish to report the results of further fluorescence spectral studies on the metabolic activation of 7-methylbenz[a]anthracene (see formula). These show that the hydrocarbon moieties, that become bound to DNA in hamster embryo cells and in mouse skin treated with this hydrocarbon, have spectral characteristics that are similar to those of DNA that has been reacted in solution with 3,4-dihydro-3,4-dihydroxy-7-methylbenz[a]anthracene 1,2-oxide, but that are different from those of DNA that has been treated with 8,9-dihydro-8,9-dihydroxy-7-methylbenz[a]anthracene 10,11-oxide.



These and other results are consistent with our general hypothesis that vicinal diol-epoxides [4] are important in the metabolic activation of the polycyclic hydrocarbons [5,6] and indicate that, with 7-methylbenz[a]anthracene, the active metabolite is most probably 3,4-dihydro-3,4-dihydroxy-7-methylbenz[a]anthracene 1,2-oxide.

2. Materials and methods

2.1. *Materials*

7-Methylbenz[a]anthracene and 3,4-dihydro-3,4-dihydroxy-7-methylbenz[a]anthracene were prepared [7,8] and 8,9-dihydro-8,9-dihydroxy-7-methylbenz-[a]anthracene was obtained from the hydrocarbon by metabolism [9]. The corresponding diol-epoxides, 3,4-dihydro-3,4-dihydroxy-7-methylbenz[a]anthracene 1,2-oxide and 8,9-dihydro-8,9-dihydroxy-7-methylbenz-[a]anthracene 10,11-oxide, were prepared from the

+ The authors would like to dedicate this paper to the memory of Mme Pascaline Daudel who died in Paris on May 12th 1976

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diols by oxidation with *m*-chloroperoxybenzoic acid in tetrahydrofuran. DNA (salmon sperm, type 111, Sigma Chemical Co., St Louis, Mo, USA) was deproteinized before use by a detergent salt-procedure [10]. 1-Methylphenanthrene and 9-methylanthracene were purchased (Aldrich Chemical Co. Gillingham, Dorset, England) and purified by chromatography on alumina and crystallization.

2.2. *Reactions with DNA*

DNA (20 mg) dissolved in Tris–HCl buffer (0.01 M, pH 7.0, 20 ml) was mixed with acetone (10 ml) or with a solution of a diol-epoxide (1 μM) in acetone (10 ml) and incubated at 37°C for 24 h. DNA solutions were then extracted and the DNA purified and reisolated as described [3] except that two additional chloroform/isoamyl alcohol extractions were performed.

DNA was also obtained from mouse skin and from cultures of hamster embryo cells that had been treated with 7-methylbenz[a]anthracene. Mouse skin DNA was isolated 24 h after the shaved dorsal areas of the skin of ♂ C57B1 mice had been treated either with acetone (0.15 ml) or with a solution of 7-methylbenz-[a]anthracene (1 μM) in acetone (0.15 ml) [3]. Hamster embryo cell DNA was isolated from cultures [5] 24 h after acetone (0.1%) or an equal volume of an acetone solution of 7-methylbenz[a]anthracene (final concentration 1 μg/ml medium) had been added to the media. Hamster embryo cell and mouse skin DNA samples were deproteinized twice [10] and the aqueous solutions were then treated briefly with RNAase and extracted repeatedly with chloroform/ isoamyl alcohol [3].

All purified DNA preparations intended for fluorescence studies were dissolved in a Tris–NaCl buffer (0.1 M, pH 7.5) prepared from water distilled from $KMnO_4$ and then from $Ba(OH)_2$ and stored in a silica container. Any particles of suspended matter, which have been found to interfere with low level fluorescence measurements, were removed from DNA solutions by centrifugation immediately prior to fluorimetry.

2.3. *Fluorescence spectra*

These were determined using a photon-counting spectrophotofluorometer of the type described [11]. However, because of the extremely low levels of fluorescence associated with DNA obtained from cells or tissues that had been treated with 7-methylbenz-[a]anthracene, the data presented (figs 1 and 2) were recorded on a more recent model of the instrument that is somewhat more sensitive. Emission spectra were recorded using excitation and emission wavelength band widths of 6 nm and of 3 nm respectively; they have not been corrected for the response of the emission path of the instrument.

3. Results and discussion

Fluorescence emission spectra recorded using the photon-counting spectrophotofluorimeter are shown in fig.1. From data of this type, difference spectra like those shown in fig.2 have been prepared. This illustration shows that the fluorescence spectrum obtained from DNA treated with the 3,4-diol 1,2-oxide derived from 7-methylbenz[a]anthracene (fig.2a, spectrum 1) is quite different from that shown by DNA that had been incubated with the

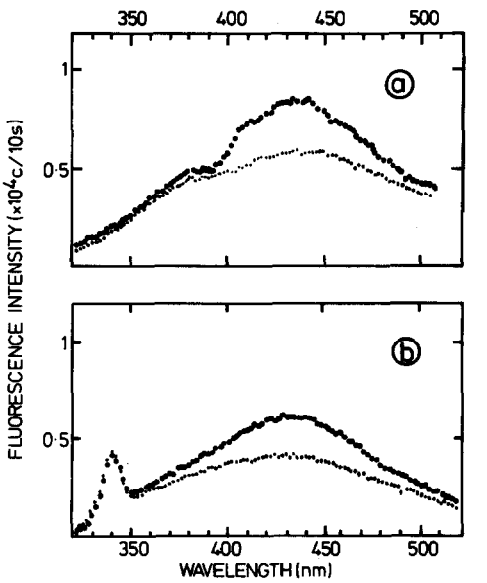

Fig.1. Fluorescence spectra shown by DNA isolated from (a) hamster embryo cells or (b) mouse skin following treatment with 7-methylbenz[a]anthracene (• • • •) or acetone (· · · ·). DNA solutions had optical densities of (a) 1.5 and (b) 15.7 at 260 nm and spectra were recorded as described in the text using the photon-counting spectrophotofluorimeter with excitation wavelengths of (a) 260 nm and (b) 300 nm.

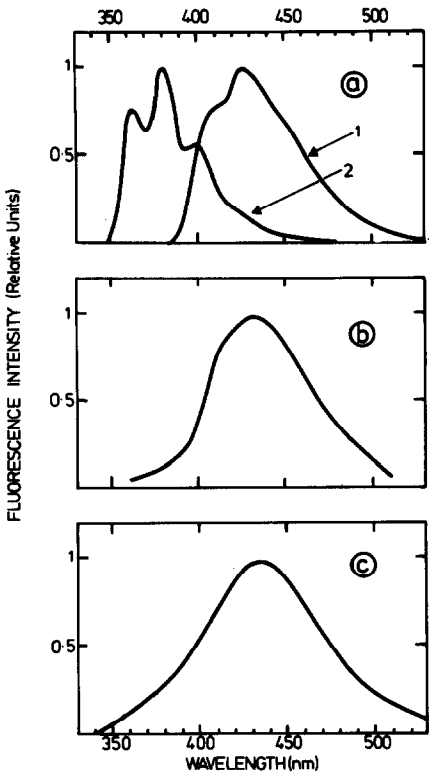

Fig.2. Fluorescence spectra shown by DNA (a) treated with 3,4-dihydro-3,4-dihydroxy-7-methylbenz[a]anthracene 1,2-oxide (curve 1) or with 8,9-dihydro-8,9-dihydroxy-7-methylbenz[a]anthracene 10,11-oxide (curve 2) or isolated from (b) hamster embryo cells or (c) mouse skin following treatment with 7-methylbenz[a]anthracene. The spectra are difference spectra normalized on their maxima obtained from samples of DNA prepared as described in the text.

related 8,9-diol 10,11-oxide (fig.2a, spectrum 2). The spectrum obtained from the 3,4-diol 1,2-oxide treated DNA closely resembles that of 9-methylanthracene, with a shift of 7.5 nm towards longer wavelengths (table 1) and that shown by the 8,9-diol 10,11-oxide treated DNA resembles, again with a similar shift, that of 1-methylphenanthrene (table 1). The difference spectra obtained from 7-methylbenz[a]anthracene-treated and control hamster embryo cell and mouse skin DNA samples are shown in fig.2b and 2c respectively.

The fluorescence was in both cases much less intense than that previously obtained in similar experiments with benzo[a]pyrene [3] but the spectra are similar to the anthracene type of spectrum shown by DNA treated with the 3,4-diol 1,2-oxide (fig.2a,1) and are obviously quite different from the phenanthrene-type spectrum displayed by DNA following reaction with the related 8,9-diol 10,11-oxide (fig.2a, 2). The fluorescence spectrum of the DNA treated with the 8,9-diol 10,11-oxide occupies the same wavelengths as that of DNA treated with the *K*-region epoxide, 7-methylbenz[a]anthracene 5,6-oxide. The emission spectrum of DNA treated with the 3,4-diol 1,2-oxide (fig.2) show a considerable loss of structure in comparison with that of 9-methylanthracene, probably due to interaction between the hydrocarbon moieties and the macromolecule, and in addition there is an important red-shift of 7.5 nm which takes the spectrum to higher wavelengths than that of 7-methylbenz[a]anthracene itself (table 1).

Previous fluorescence and other studies [1,12]

Table 1

	Fluorescence maxima (nm)[a]				
7-Methylbenz[a]anthracene	398	420	444	471.5	
9-Methylanthracene	397	417.5	442	470	495
DNA treated with 3,4-dihydro-3,4-dihydroxy-7-methylbenz[a]-anthracene 1,2-oxide	407 (shoulder)	425	455 (shoulder)		
1-Methylphenanthrene	356.5	373	392.5	412.5	440
DNA treated with 8,9-dihydro-8,9-dihydroxy-7-methylbenz-[a]anthracene 10,11-oxide	362.5	380	400	420	

[a] The spectra of the hydrocarbons were measured in ethanol and those of the DNA samples in buffer as described in the text

have helped to exclude the *K*-region epoxide and carbonium ions formed on the methyl group from those active species that may be formed when 7-methylbenz[a]anthracene is metabolized in vivo. The results presented here appear to exclude a vicinal diol-epoxide formed on the 8,9- and 10,11-double bonds from this activation process but are not inconsistent with the formation of a biologically important vicinal diol-epoxide in the 1,2,3,4-positions; further studies are, however, required to prove this conclusively. Although the 1,2-diol has not been described as a metabolite of 7-methylbenz[a]anthracene, the 3,4-diol has been detected [13]. When tested in bacterial and mammalian cell mutagenicity test systems and in an in vitro malignant transformation system, which are all systems where further metabolism can occur, the 3,4-diol derived from 7-methylbenz[a]anthracene has been found to be more active than either the parent hydrocarbon or other dihydrodiols [14,15]. In addition, in experiments that are still in progress, this diol is proving to be a potent mouse skin tumour initiating agent [16]. In total therefore the information now available strongly suggests that the metabolic activation of 7-methylbenz[a]anthracene involves the formation of the vicinal diol-epoxide 3,4-dihydro-3,4-dihydroxy-7-methylbenz[a]anthracene 1,2-oxide.

Acknowledgements

We wish to thank Professor R. Daudel for the interest that he has shown in this work and to acknowledge the skilled technical assistance of Joelle Tanguy, Christine Walsh and Alan Hewer. The investigations have been supported by grants to the Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, from the Medical Research Council and the Cancer Research Campaign and to the Laboratoire Curie from the Délégation Générale à la Recherche Scientifique et Technique.

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