FLUORESCENCE SPECTRAL EVIDENCE THAT BENZO(*a*)PYRENE-DNA PRODUCTS IN MOUSE SKIN ARISE FROM DIOL-EPOXIDES

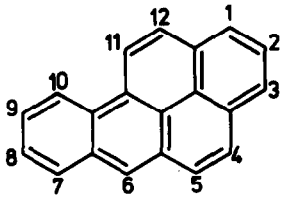
Pascaline DAUDEL, Maurice DUQUESNE, Paul VIGNY, Philip L. GROVER* and Peter SIMS*

*Institut du Radium, 11, Rue Pierre et Marie Curie, 75231 Paris, Cedex 05, France, *Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW3 6JB, England*

Received 26 June 1975

1 . Introduction

When carcinogenic polycyclic hydrocarbons like benzo(*a*)pyrene (I) are metabolized, derivatives are formed that react with cellular macromolecules [1,2]. The positive identification of the reactive metabolites concerned has been hindered by the very low levels of reaction with nucleic acids that occur in tissues treated with these chemical carcinogens, although some progress has been made using radioactively-labelled hydrocarbons [3]. A fresh approach to this problem has been made possible by the construction of a highly sensitive spectrophotofluorometer that incorporates a photon-counting device [4]. The instrument allows the fluorescence characteristics of polycyclic hydrocarbon-modified nucleic acids that have been isolated from treated tissues to be investigated directly without prior degradative or chromatographic procedures [5]. We report here the results of the first studies on benzo(*a*)pyrene undertaken with this spectrophotofluorometer in which the fluorescence spectral characteristics of DNA from benzo(*a*)pyrene-treated mouse skin have been compared with those of DNA treated in solution with reactive benzo(*a*)-pyrene derivatives.



I

2. Materials and methods

2.1. *Materials*

Benzo(*a*)pyrene, purified by recrystallization from xylene, and DNA (salmon sperm, type III), deproteinized by a detergent salt procedure [6], were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. *m*-Chloroperoxybenzoic acid was obtained from BDH Chemicals Ltd., Poole, Dorset, U. K. 7,8-Dihydro-7,8-dihydroxybenzo(*a*)pyrene and 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene were obtained from the hydrocarbon by metabolism [7]; the corresponding diol-epoxides, 7,8-dihydro-7,8-dihydroxybenz(*a*)pyrene 9,10-oxide and 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene 7,8-oxide were prepared from the diols by oxidation with *m*-chloroperoxybenzoic acid in benzene. Benzo(*a*)pyrene 4,5-oxide was also synthesized [8].

2.2. *Reactions with DNA*

DNA (10 mg) dissolved in Tris-HCl buffer (0.1M, pH 7.0, 10 ml) was mixed with acetone (5 ml) or with a solution of a benzo(*a*)pyrene epoxide (200 μg) in acetone (5 ml) and incubated for 18 h at 37°C. After incubation and extraction with ether (2 x 1 vol.), NaCl ($\cong$ 1 M) was added and DNA reprecipitated with ethanol and purified by passage through G10 Sephadex and extraction with chloroform: isoamyl alcohol (24 : 1, 1 vol.). After concentration by lyophilization, NaCl was added ($\cong$ 1 M), the DNA was reprecipitated with ethanol (2 vol.) and then washed extensively with ethanol. DNA was finally redissolved in Tris-NaCl buffer (0.01 M, pH 7.4) and

North-Holland Publishing Company – Amsterdam

dialysed (48 h) against this buffer (2000 vol.) before fluorescence spectra were recorded. DNA was also isolated from mouse skin following treatment with benzo(*a*)pyrene. The backs of groups of 15 male C57Bl mice were shaved and treated with acetone (0.15 ml) or with benzo(*a*)pyrene (250 μg) in acetone (0.15 ml). After 24 h the mice were killed, the treated skin removed, frozen in liquid N_2 and the dermal surface scraped with a scalpel. The frozen epidermal layers were cut into fragments and homogenized in liquid N_2 using an Ultra-Turrax (Janke and Kunkel, K. G., Staufen, Germany). DNA, isolated as described [9], was further purified as described above.

2.3. *Fluorescence spectra*

These were obtained using the photon-counting spectrophotofluorometer [4]. The points plotted by the instrument (fig.1) show the fluorescence emitted by the sample at that wavelength in 10 s using solutions of DNA treated with 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene 9,10-oxide. Excitation wavelength 302.5 nm with $\Delta\lambda_0$ = 4.2 nm. Band width of the analysis path $\Delta\lambda_i$ = 6.4 nm; concentration of DNA, 700 μg/ml. All the spectra shown in fig.2 are difference spectra where the fluorescence contribution of the relevant control DNA has been subtracted; these were obtained with the instrument settings given above.

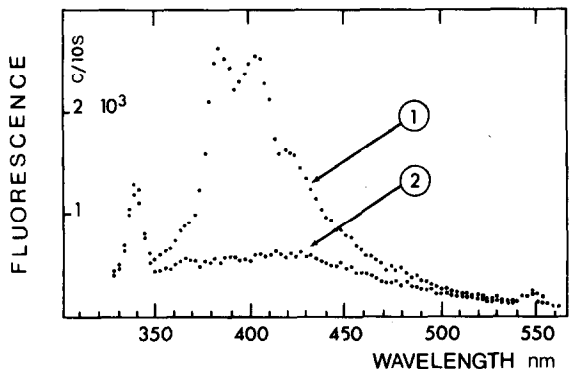


Fig.1. Fluorescence spectra shown by 1, DNA reacted with 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene 9,10-oxide and 2, DNA, both recorded using the photon-counting spectrophotofluorometer. Measurements were made as described in the text.

3. Results and discussion

Since evidence that implicated 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene 9,10-oxide in the metabolic activation of benzo(*a*)pyrene existed [7], the fluorescence spectrum of DNA that had been reacted with this diol-epoxide was compared with that of benzo(*a*)-pyrene-treated mouse skin DNA. Fig2(c) shows that the main fluorescence characteristics of DNA isolated from the hydrocarbon-treated mouse skin very closely resemble those of salmon sperm DNA treated with the 7,8-diol 9,10-epoxide. Apart from the characteristics contributed by the diol-epoxide (fig.2(a) I), spectral contributions from two other components (fig.2(a), II and III), which have not been identified, also appear to be present in DNA isolated from benzo(*a*)pyrene treated mouse skin. Figure2(b) shows the fluorescence spectra of salmon sperm DNA reacted with benzo(*a*)pyrene 7,8-diol 9,10-epoxide or with the isomeric 9,10-diol 7,8-epoxide; these cannot be distinguished from one another and both show maxima at 382.5 and 402 nm. Other evidence indicates, however, that the 9,10-diol 7,8-epoxide is not involved in the metabolic activation of benzo(*a*)pyrene in vivo [7]. Earlier work had shown that simple K-region epoxides derived from polycyclic hydrocarbons possessed properties relevant to carcinogenesis [10] and that benzo(*a*)pyrene 4,5-oxide was formed as a microsomal metabolite [11]. The DNA products that are formed, however, when benzo(*a*)pyrene is metabolized in vivo do not seem to result from reaction with this K-region epoxide [7,12]. The fluorescence spectral studies carried out using DNA treated with benzo(*a*)pyrene 4,5-oxide confirm this; the fluorescence maxima for the K-region epoxide-reacted DNA, at 371.5 and 390 nm (fig.2(c)), are clearly different from those of benzo(*a*)pyrene-treated mouse skin DNA and of diol-epoxide reacted DNA. Fig.2(c) also gives, for comparative purposes, the fluorescence spectrum of benzo(*a*)pyrene itself, which is quite distinct from the others obtained.

The use of the photon-counting spectrophotofluorometer has permitted the fluorescence emitted by benzo(*a*)pyrene derivatives that have become bound to DNA in vivo to be examined for the first time. The spectra obtained show that the main benzo(*a*)pyrene derivative bound to the DNA of mouse skin, a tissue in which benzo(*a*)pyrene is car-

cinogenic, retains an intact pyrene nucleus (fig.2). This indicates that metabolism of the 7,8,9,10-ring precedes reaction with DNA and is consistent with the hypothesis that a diol-epoxide formed on this ring is involved [7]. The spectra do not permit the major metabolite concerned to be characterized further, however, since both isomeric benzo(*a*)pyrene diol-epoxides give similar fluorescence spectra after reaction with DNA. It has been suggested that free radicals [13], radical cations [14] and hydroxymethyl derivatives [15], formed from benzo(*a*)pyrene, may react with DNA in vivo; these types of intermediates could conceivably be involved in the formation of the unidentified fluorescent component (fig.2(a), III) that is present in benzo(*a*)pyrene-treated mouse skin DNA but more information would be necessary to show this. The results which we have obtained with benzo(*a*)pyrene and those reported for 7-methylbenz(*a*)anthracene [5] demonstrate the high sensitivity of this photoncounting spectrophotofluorometer. One important advantage of this instrument is that it allows polycyclic hydrocarbon derivatives that become bound to nucleic acids in vivo to be examined without the need to synthesise radioactively-labelled compounds. Since the instrument also provides the only available means of characterizing these hydrocarbon residues, it is intended to apply the spectrophotofluorometer to other carcinogenic aromatic hydrocarbons whose metabolic activation is being investigated.

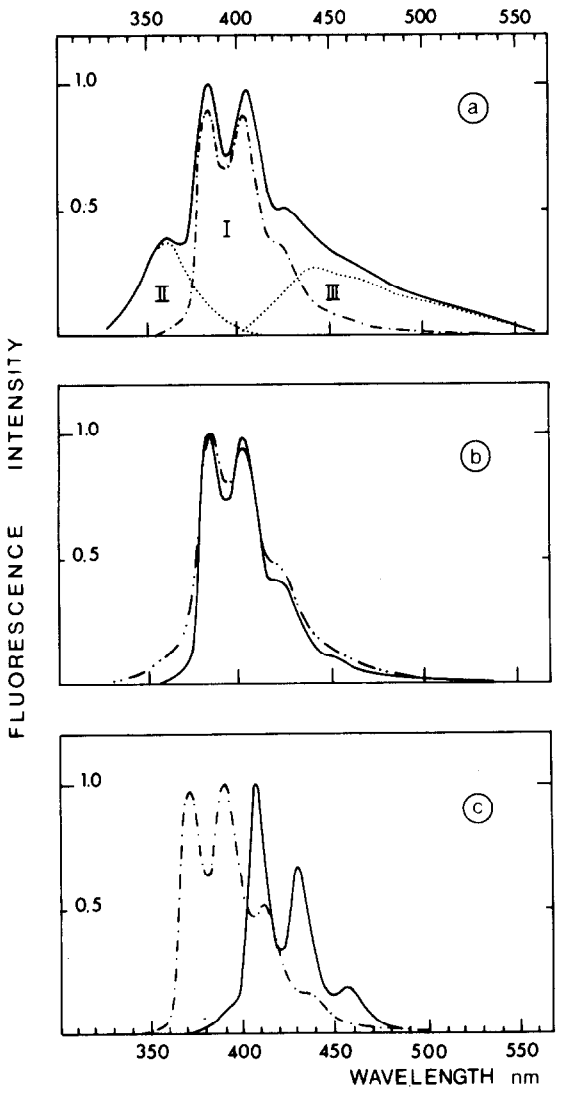


Fig.2. Fluorescence spectra shown by (a) ————, DNA isolated from mouse skin following treatment with benzo(*a*)pyrene. .–.–.–.–.–, DNA treated with 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene 9,10-oxide. (b) ————, DNA reacted with 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene 9,10-oxide. –..–..–..–..–.., DNA reacted with 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene 7,8-oxide. (c) ————, benzo(*a*)pyrene, measured as a solution in methanol. –.–.–.–.–., DNA reacted with benzo(*a*)pyrene 4,5-oxide. Measurements were made as described in the text.

Acknowledgements

This research was 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. We thank I. Chiron de la Casiniere, A. Hewer, L. Lacombe, C. Alonso-Verduras and J. Tanguy for technical assistance.

References

[1] Grover, P. L. and Sims, P. (1968) Biochem. J. 110, 159–160.
[2] Gelboin, H. V. (1969) Cancer Res. 29, 1272–1276.
[3] Baird, W. M., Dipple, A., Grover, P. L., Sims, P. and Brookes, P. (1973) Cancer Res. 33, 2386–2392.
[4] Vigny, P. and Duquesne, M. (1974) Photochem. Photobiol. 20, 15–25.

[5] Daudel, P., Croisy-Delcey, M., Alonso-Verduras, C., Duquesne, M., Jacquignon, P., Markovits, P. and Vigny, P. (1974) C. R. Acad. Sci. 278, 2249–2252.
[6] Kay, E. R. M., Simmons, N. S. and Dounce, A. L. J. (1952) J. Am. Chem. Soc. 74, 1724–1726.
[7] Sims, P., Grover, P. L., Swaisland, A., Pal, K. and Hewer, A. (1974) Nature 252, 326–328.
[8] Goh, S. H. and Harvey, R. G. (1973) J. Am. Chem. Soc. 95, 242–243.
[9] Grover, P. L., Forrester, J. A. and Sims, P. (1971) Biochem. Pharmacol. 20, 1297–1302.
[10] Sims, P. and Grover, P. L. (1974) Adv. Cancer Res., 20, 165–274.
[11] Grover, P. L., Hewer, A. and Sims, P. (1972) Biochem. Pharmacol. 21, 2713–2726.
[12] Baird, W. M., Harvey, R. G. and Brookes, P. (1975) Cancer Res. 35, 54–57.
[13] Hoffman, H. D., Lesko, S. A. and Ts'o, P. O. P. (1970) Biochemistry 9, 2594–2604.
[14] Wilk, M. and Girke, W. (1972) J. Natl. Cancer Inst. 49, 1585–1597.
[15] Flesher, J. W. and Sydnor, K. L. (1973) Int. J. Cancer 11, 433–437.