Specificity of Interaction Between Carcinogenic Polynuclear Aromatic Hydrocarbons and Nuclear Proteins: Widespread Occurrence of a Restricted Pattern of Histone-binding in Intact Cells.

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Research supported by grant #RO1 CA303550-01 from the National Cancer Institute and by the Office of Health & Environmental Research, U. S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.

It is widely accepted that the first steps in the induction of malignancy by chemical carcinogens involve covalent interactions with cellular macromolecules. For the widespread environmental contaminant benzo(a)pyrene [B(a)P], metabolic activation by cellular enzymes produces a number of potentially reactive metabolites (1,2). The endproducts of one metabolic pathway, 7,8-dihydroxy-9,10-oxy-7,8,9,10-tetrahydro-B(a)P (BPDE) are responsible for essentially all DNA adduct formation in animal cells treated with B(a)P (3-5), and a particular stereoisomer, designated (+)-anti-BPDE is thought to be the ultimate carcinogenic derivative of B(a)P (6,7). We have recently shown (8,9) that in hamster embryo cell nuclei treated with (+)-anti-BPDE, two of the histones of the nucleosomal core, H3 and H2A, are covalently modified, while the remaining core histones, H4 and H2B, are essentially unmodified. All four purified core histones, however, serve as targets for (+)-anti-BPDE *in vitro* (10). The restricted histone-binding pattern seen in hamster embryo cell nuclei is the same as that seen in intact hamster embryo cells treated with B(a)P or 7,8-dihydroxy-7,8-dihydro-B(a)P (8), the metabolic precursor of BPDE. Several lines of evidence (8,9,11) led us to suggest that the restriction of histone binding seen in intact nuclei is a reflection of constraints imposed by the structure of chromatin. Interestingly, two structurally different polycyclic aromatic hydrocarbons, 7,12-dimethylbenz(a)anthracene and 3-methylcholanthrene show the same pattern of histone binding in hamster embryo cells (11).

The present series of experiments demonstrates that the restricted histone binding pattern is not unique to hamster embryo cells, but occurs in cells derived from other species and in nonfibroblastic cells. Treatment of mouse embryo cells with [^{3}H]-BPDE results in covalent binding of the hydrocarbon to cellular macromolecules. As shown in Figure 1, histones H3 and H2A are among

the many cellular targets for BPDE-binding while histones, H2B and H4 are not. Similar binding patterns are seen in mouse embryo cells treated with [^{3}H]-B(a)P for periods from 8-96 hr and in a permanent murine, fibroblastic cell line, NIH-3T3 (manuscripts in preparation).

To further probe the generality of the restricted histone binding pattern, we have studied a human mammary epithelial cell line, T47D, incubated with [^{3}H]-B(a)P. As shown in Figure 2, after 12 or 24 hr of exposure, nuclear proteins have become labeled by derivatives of the radioactive carcinogen. Again, the histones are unevenly labeled, displaying the H3 and H2A pattern of specificity seen in murine and hamster cells. By analogy with the results in rodent cell systems, it seems likely that histone-binding in the human cells is also mediated by BPDE. Similar BPDE binding patterns have recently been observed in other murine and human cell lines (A. Kootstra, personal communication) and in primary cultures of murine epidermal epithelial cells (JCP and TJS, unpublished observations). The restriction of histone H2B and H4 binding thus appears to be a relatively general finding when intact cells in culture are studied, and this specificity is maintained in isolated hamster embryo cell nuclei treated with BPDE (8). In contrast, this specificity was not observed in a mixed reconstituted system in which rat liver microsomes were used to activate B(a)P and binding was monitored in isolated calf thymus nuclei (15). This finding reinforces reservations previously expressed (16-18) concerning the use of microsomal systems to probe the interactions of carcinogens with macromolecules and the relationships of adduct formation with the processes of carcinogenesis.

Figure Legends

Fig. 1. Binding of BPDE to Nuclear Proteins in Mouse Embryo Cells. Confluent, tertiary cultures of fibroblastic cells were prepared from 18d embryos of SENCAR mice as described (12) and treated for 1 h with [^{3}H]-(±)-anti-BPDE (1.0 µg/ml, 510 Ci/mmol; NCI Chemical Repository). Nuclear proteins were prepared and analyzed by SDS-polyacrylamide gel electrophoresis as described (8,9,12,13). The gels were stained with Coomassie Blue R (lane S) and then subjected to fluorography (lane F) to determine the distribution of [^{3}H]-BPDE among the nuclear proteins.

Fig. 2. Binding of B(a)P to Nuclear Proteins in Human T47D Mammary Carcinoma Cells.

T47D cells, an epithelial-like cell line derived from a patient with mammary carcinoma, were grown to confluence in medium RPMI 1640 containing 10% fetal bovine serum and 0.2 IU/ml insulin (14). Confluent cell cultures were exposed to [^{3}H]-B(a)P (1 µg/ml; 6.0 Ci/mmol; Amersham Radiochemicals, Arlington Heights, IL; radiochemical purity >95%) for 12 or 24 hr to allow cellular metabolism of the carcinogen. Further analysis was as described for Fig. 1.

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Figure 1

Figure 2