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BINDING OF POLYCYCLIC AND NITROPOLYCYCLIC AROMATIC HYDROCARBONS TO SPECIFIC FRACTIONS OF RAT LUNG CHROMATIN

Abstract — Binding of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons (NPAH) to rat lung nuclei was investigated. Following carcinogen exposure, nuclei were fractionated into active chromatin, nuclear matrix, low salt, and high salt fractions. Preferential binding to active chromatin and nuclear matrix fractions was observed for benzo(a)pyrene (BP), 6-nitro benzo(a)pyrene, 1,6-dinitropyrene (1,6-DNP), and 1-nitropyrene. Incubation of nuclei with BP, benzo(a)pyrene diolepoxide (BPDE), and 1,6-DNP showed that the selective binding was dependent upon the concentration of chemical with less selectivity at higher concentrations. This study shows that NPAH should be considered as another class of compounds that may exert their biological effects by binding to selected regions of chromatin that are involved in DNA replication and translation.

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There is evidence that the organization of DNA into the complex and dynamic structure of chromatin has significant consequences for the efficiency of damage recognition and removal. In nuclei, chromatin is anchored to a structural protein matrix that is present in a variety of eukaryotic cell types and is left behind when nuclei are extracted with solutions of detergent containing a high salt concentration.[1] In addition to acting as a structural framework within the nucleus, the matrix appears to provide a platform for transcription and replication, and may represent another potential level of control for regulation of gene expression in the eukaryotic cell.[2] Since the nuclear matrix has been claimed as the site of replication, transcription and DNA repair, carcinogen-induced damage to matrix DNA could have profound effects in a cell. It has been shown that DNA damage is not uniform throughout the genome, and that repair is more efficient in some regions of the genome than in others.[3] Heterogeneity in the initial binding and subsequent repair would result in corresponding differences in particular biological effects, since the consequences of unrepaired or misrepaired damage in DNA clearly depend upon the precise location of the damage in the genome.

Relatively few chemicals have been investigated for their abilities to bind to nuclear matrix and chromatin fractions. Essentially all of the work has been done with polycyclic aromatic hydrocarbons (PAH) and their binding to rat liver nuclei where it was shown that differential binding occurs throughout the genomic DNA, and that chemical specificity may exist with respect to where binding occurs.[3] Nitropolycyclic hydrocarbons (NPAH) are another ubiquitous class of environmental pollutant that are also potent mutagens, and cause cancer in animals.[4] However, there is no information on the structure-function relationships relative to their binding to selected regions of DNA hierarchy structures. The current study was undertaken to determine the intragenomic binding regions of a series of NPAH and benzo(a)pyrene (BP) to rat lung nuclei.

METHODS

Groups of 10 adult male Fischer 344/N rats, 8-10 wk of age, were pretreated with an intraperitoneal injection of 3-methycholanthrene (50 mg/kg) in corn oil 48 h before sacrifice to

induce arylhydrocarbon hydroxylase activity. The rats were sacrificed by CO_2 asphyxiation and the lungs were removed and homogenized, using a tissumizer, in 10 volumes of Tris-HCl buffer (10 mM, pH 7.4) containing $MgCl_2$ (10 mM), sucrose (0.25 M) and 2-mercaptoethanol (0.03 mM) (TMS). The homogenate was filtered through gauze and mixed with an equal part of TMS containing 2.0 M sucrose. The nuclei were isolated by dividing the homogenate into four aliquots and layering each over portions (13 mL) of modified sucrose (2.0 M in centrifuge tubes). The centrifuge tubes were spun at 45,000 rpm for 45 min at 4°C in the SW-28 swing-out rotor using an L5-75 ultracentrifuge. The pelleted nuclei were used immediately.

Nuclei were incubated in TMS that also contained EDTA (1 mM) and NADPH (5 mM) for 30 min at 37°C in the presence of ^3H-labeled benzo(a)pyrene (BP), benzo(a)pyrene diolepoxide (BPDE), 1-nitropyrene (1-NP), 1,6-dinitropyrene (1,6-DNP), and 6-nitrobenzo(a)pyrene (6-NBP) (0.4-40 μM) that were added as solutions in DMSO (final concentration 1% v/v). After incubation with each carcinogen, micrococcal nuclease (75-150 U/mL) was added and the incubation continued for an additional 5 min to release the active chromatin (AC) fraction. The reaction was stopped with ethylene glycol-bis(β-aminoethylether)N,N,N',N'-tetraacetic acid (EGTA). The suspension was centrifuged at 800 g for 10 min and the supernatant (AC) was removed and saved. The pellet was resuspended in Tris-HCl buffer (10 mM, pH 7.4) containing $MgCl_2$ (0.2 mM), left on ice for 10 min and pelleted at 1000 g for 15 min. This procedure was repeated once and the low salt (LS) chromatin supernatants were combined for analysis. The remaining nuclear pellet was further extracted with a high salt (HS) buffer (2.0 M NaCl, 10 mM Tris, pH 7.4, and 0.2 mM $MgCl_2$) in a similar fashion. HS supernatants were combined for analysis. The high salt-resistant pellet was then rinsed once with 1% Triton-X 100 in LS buffer, and then again with LS buffer alone. The supernatants were then concentrated by adding two volumes of ethanol and leaving overnight at -20°C. The DNA was extracted and purified, as described previously (1979-80 Annual Report, LMF-84, pp. 455-457). The DNA was quantitated by the absorbancy at 260 nm, and covalently bound radioactivity was determined by scintillation spectrometry. The quantity of labeled compound bound to DNA was calculated from the specific activity of the labeled compound and expressed as femtomoles of covalently-bound chemical per microgram of DNA.

RESULTS

Figures 1A-1D show the binding of BP, 6-NBP, 1-NP, and 1,6-DNP to chromatin fractions after incubation of carcinogens with rat lung nuclei. The highest binding occurred with the active chromatin and nuclear matrix fractions for all of the carcinogens investigated. The highest ratios for the binding to active chromatin and nuclear matrix relative to the LS and HS fractions ranged from 1.5 to 11 depending upon the compound and the fraction. Individually, 1-NP had the highest differential binding, although this compound gave the lowest total binding overall.

Figure 2 shows the ratio of the binding of active chromatin and nuclear matrix to bulk DNA (LS and HS) as dependent on the concentration of BP, BPDE, and 1,6-DNP. The binding to specific fractions were affected by the concentration of BP and 1,6-DNP. At low concentration (0.4 μM) high binding occurred to the active chromatin and nuclear matrix fractions, but as the concentration of compounds were increased the binding became random and was essential equal in all fractions at carcinogen concentrations of 4-40 μM. In contrast, BPDE (an ultimate direct acting carcinogen, i.e., requires no metabolism prior to binding to DNA) bound randomly to DNA fractions at concentrations ranging from 0.4 to 40 μM.

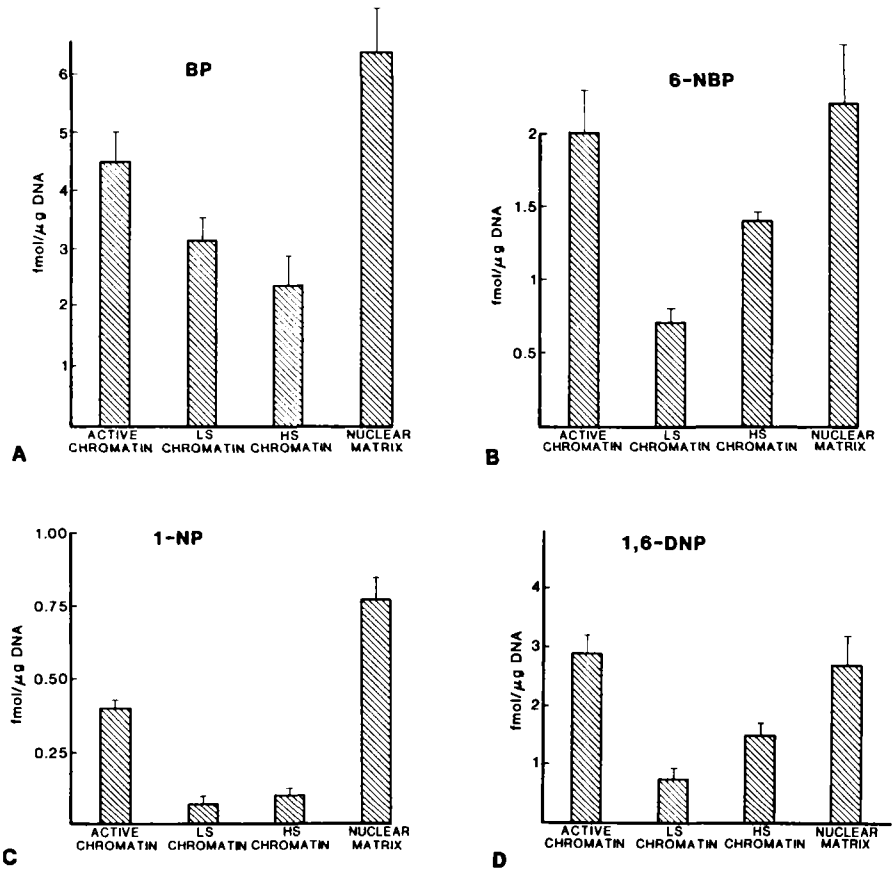

Figure 1A-1D. Covalent binding of BP, 6-DNP, 1-NP, and 1,6-DNP to DNA of subnuclear fractions. Bars represent the mean ± SD of three measurements.

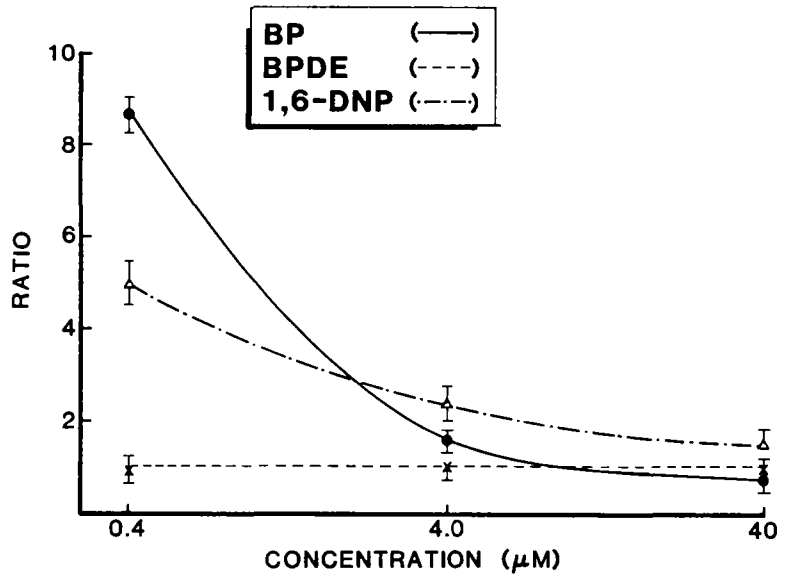

Figure 2. The effect of BP, BPDE, and 6-NBP concentration on their binding to subnuclear fractions. The ratio is the binding of carcinogens to active chromatin and nuclear matrix divided by the binding to low and high salt fractions. Bars represent the mean ± SD of three measurements.

DISCUSSION

A number of compounds have been investigated for their ability to bind to selected regions of chromatin and subsequently affecting normal cellular functions. However, NPAH have not been investigated for their ability to bind to selected regions of chromatin. It was shown in the present study that NPAH bound nonuniformly to selected regions of chromatin with the highest binding occurring to the active chromatin and nuclear matrix regions. The series of nitro compounds investigated were similar in that they all contain nitro groups; however they were dissimilar in the fact that they may be metabolized differently (e.g., monooxidation, nitroreduction or a combination of the two) to metabolites that may be detoxified and removed from the cell or activated to a metabolite that readily binds to macromolecules.[5] Our data indicated that the nature of the metabolites was such that they showed preferential binding for the same specific fractions. Thus, the binding was not dependent upon a given structure of this series of NPAH investigated.

When the binding of three compounds, BP, BPDE, and 1,6-DNP, were determined relative to their concentration, a definite dose-dependency was observed for BP and 1,6-DNP. BPDE (the ultimate carcinogen) showed no preferential binding at the concentrations investigated. Our studies with BPDE are in agreement with others[6] and suggest that with low BPDE concentrations, the diolepoxide would react with DNA and protein in close proximity to the nuclear membrane (i.e., in nuclear matrix) before it was hydrolyzed. When, however, high concentrations were applied, sufficient BPDE might have diffused into the nucleoplasm to modify all forms of nuclear DNA more uniformly, as indeed appeared to be the case. We are currently examining BPDE binding at much lower concentrations to determine at what concentration preferential binding occurs to rat lung nuclear DNA fractions.

In assessing the risks from exposure to carcinogens, it has been suggested that binding of carcinogens to selective DNA regions and repair of those regions may be more important than binding to total DNA. This study and others suggest that it may be even more important to obtain binding and repair data on the active chromatin and nuclear matrix fractions. Considering the probable human exposure to low amounts of carcinogens, carcinogens such as BP and NPAH can be expected to bind with greater selectivity toward active chromatin and nuclear matrix. In conclusion, this study indicates that the NPAH is another class of environmental pollutants that show the ability to bind to selected regions of chromatin. The cellular effects of NPAH (mutagens, carcinogens, cell transformants, induction of protooncogenes) may be the result of their binding to regions actively involved in cell replication and DNA transcription.

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