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BASE SPECIFICITY IN THE BINDING OF BENZO[A]PYRENE DIOL EPOXIDE
TO SIMIAN VIRUS 40 DNA

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Summary: [^{14}C] Simian virus 40 DNA was reacted with [^3H]-7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene to give 0.60 adducts per genome. The modified DNA was digested to completion with Hind III restriction endonuclease and the six fragments isolated by polyacrylamide gel electrophoresis. Hydrocarbon binding to the fragments was proportional to their guanine-cytosine content, reflecting selective reaction of the hydrocarbon with deoxyguanosine residues. No sites unusually susceptible to alkylation were detected.

The polycyclic aromatic hydrocarbon benzo[a]pyrene, a prevalent environmental carcinogen, is metabolized in vivo by microsomal monooxygenases to reactive mutagenic and carcinogenic intermediates which bind covalently to cellular macromolecules (1-3). It has recently been suggested that BP-diol epoxide is the species responsible for binding to nucleic acids and that this intermediate may be the ultimate carcinogenic metabolite (4).

The primary site of BP-diol epoxide alkylation in DNA is the exocyclic amino group of guanine (5-9). Intercalation and orientation of the hydrocarbon within the helix may precede covalent linkage and account for the selectivity in alkylation. Polycyclic aromatic hydrocarbons exhibit very low levels of intercalation. Lesko et al. (10) have shown that less than two molecules of BP are intercalated per 10^3 base pairs under saturating in vitro conditions. Adduct formation in vivo occurs to an even lesser extent, with one BP residue incorporated per 10^4 base pairs (11). The low frequency of covalent binding coupled with probable intercalation suggests that in DNA the reaction of BP-diol epoxide with guanine might be modulated by the primary as well as secondary structure of the duplex.

We have utilized the SV40 DNA molecule, consisting of 5200 base pairs, in assessing the effect of DNA structure on BP-diol epoxide binding. After reacting SV40 DNA with BP-diol epoxide, the modified DNA was treated with Hind III restriction endonuclease to generate a set of six fragments. Alkylation of these fragments was correlated with their G-C content, implying that binding is largely unaffected by higher order DNA structure. The G-C correlation is consistent with selective reaction of BP-diol epoxide with deoxyguanosine.

Materials and Methods

The TC-7 line of African green monkey kidney cells, obtained from Dr. James Robb of the University of California at San Diego, was grown in a CO₂ incubator in 100 x 20 mm Falcon Tissue Culture Dishes in Eagle's medium as modified by Dulbecco (Gibco) with 10% calf serum (Flow Laboratories). Wild type SV40 small plaque strain Rh911 was a generous gift from Dr. Robert Su of the Harvard Medical School. Confluent TC-7 cells were infected with SV40 at 10-20 PFU per cell, and 18 hr after infection 15 µCi of [¹⁴C] thymidine was added per 100 mm plate. Cells were lysed 42 hr postinfection by the method of Hirt (12). The Hirt supernatant was extracted twice with equal volumes of Tris-buffered (10 mM Tris-HCl, 1 mM EDTA, pH 7.9) phenol. The DNA was precipitated by ethanol addition, redissolved, and centrifuged to equilibrium in a CsCl/ethidium bromide gradient. The FI band was collected, extracted with salt saturated butanol, and dialysed overnight against Tris buffer. The labeled DNA was diluted with cold carrier and had a specific activity of 3,600 dpm/µg DNA.

[³H] BP-diol epoxide (uniformly-labeled, 1.3C./mmole) was synthesized by Dr. Kenneth Straub essentially by the method of McCaustland et al. (13). The stock solution (1.6 mg/ml in 19:1 tetrahydrofuran-triethylamine) was stored at -70°C and dilutions made with DMSO. BP-diol epoxide was hydrolyzed to BP-tetraol in 0.1 N HCl at 37°C for 2 hr. The BP-tetraol was extracted into ethyl acetate, lyophilized, and dissolved in DMSO.

The reaction mixtures, containing 2.5 µg of [¹⁴C] SV40 DNA and 0.005-0.05 µg [³H] BP-diol epoxide or [³H] BP-tetraol, were incubated 24 hr at 37°C in 100 µl of 20 mM Tris-HCl buffer, pH 8.0, with 0.5 mM EDTA and 5% DMSO. After reaction, free hydrocarbon was removed by two equal volume ethyl acetate extractions. The BP modified DNA was digested to completion with 10 units of Hind III restriction endonuclease (Miles Laboratories) in 20 mM Tris-HCl buffer, pH 7.9, with 7 mM MgCl₂ and 60 mM NaCl at 37°C for 10-12 hr. This enzyme cleaves SV40 DNA into six fragments with base pair totals of 1781, 1165, 1067, 528, 444, and 217 and percent G-C contents of 40.4, 36.0, 47.0, 36.9, 40.3, and 42.4, respectively (14).

Prior to electrophoresis the DNA samples were adjusted to 0.5% SDS, 2.5% glycerol, and 0.0025% bromphenol blue. FI DNA was isolated by 1.4% agarose slab gel electrophoresis (15) immediately after ethyl acetate extraction. The Hind III restriction fragments were separated by 2% polyacrylamide-0.5% agarose slab gel electrophoresis (16). The gels were stained with ethidium

bromide and the fluorescent bands excised under ultraviolet light. Samples of blank gel vertically adjacent to the DNA bands were also excised to estimate background ^{14}C and ^3H counts. Both DNA bands and their blanks were dried overnight at 37°C , combusted, and counted. In the case of Hind III fragments, ^{14}C counts were corrected to reflect the A-T content of the entire SV40 DNA molecule, i.e., 59.5%.

Results and Discussion

Fig. 1 shows that binding of BP-diol epoxide to SV40 DNA is proportional to the molar ratio of hydrocarbon to DNA in the reaction mixture. Most of this binding reflects covalent adducts, although up to 15% may be intercalated BP-tetraol which survives ethyl acetate extraction and gel electrophoresis. BP-diol epoxide has been shown to cause single-strand cleavage of DNA (17). However, at the low ratios of BP-diol epoxide to DNA used here less than 5% of the FI DNA was relaxed.

In subsequent experiments SV40 DNA was reacted with BP-diol epoxide at a molar ratio of 2.5×10^{-3} , thereby introducing 0.60 adducts per molecule of DNA. This level of adduct formation is approximately that seen in vivo (11), and had no effect on Hind III digestion. Fig. 2 shows the distribution of adducts among the six Hind III fragments. These are average values from at least five independent experiments. The extent of adduct formation is not strictly dependent upon DNA length, and this appears to reflect variability in the G-C content of the fragments. Those fragments with high G-C content lie above the line, while those with low G-C content lie below.

By normalizing the fragment length to 10^3 base pairs, the effect of G-C content on BP-diol epoxide binding can be examined (Fig. 3). Within the limited range covered, binding is clearly dependent upon G-C content. A least squares fit of the average values in Fig. 3 to linear and exponential curves gives correlation coefficients of 0.93 and 0.94, respectively. Extrapolation of these curves to the homopolymer limits shows

that over 95% of the alkylation is restricted to G-C base pairs. These results agree with several adduct analysis studies, which demonstrate selective reaction of BP-diol epoxide with deoxyguanosine residues in DNA (5-9).

It must be emphasized that the exact relationship between BP-diol epoxide binding and G-C content can only be determined by an extension of the data over a wider G-C range. Restriction of the SV40 genome into fragments with still fewer base pairs whose percent G-C contents lie outside the 35-50% range was not attempted in light of the high standard deviation associated with the smallest Hind III fragment. Larger fragments with significantly higher or lower percent G-C contents cannot be generated from SV40 DNA.

Evidence suggests that repetitive G-C sequences may facilitate intercalation and covalent binding of BP-diol epoxide to DNA. Warshawsky (18) has shown that the efficiency with which a variety of synthetic and naturally occurring nucleic acids solubilize BP through intercalation is a function of their guanine content. Repetitive G-C sequences are also highly susceptible to frameshift mutation by aromatic carcinogens (19), a process which is most likely initiated by intercalation and covalent linkage of the carcinogen to DNA. Local base pair specificity in the interaction of BP-diol epoxide with DNA would lead to a nonlinear (perhaps exponential) relationship between binding and G-C content.

Despite the low level of modification, no reaction "hotspots" were detected. The distribution of adducts among the Hind III fragments was solely dependent upon their length and G-C content. This excludes any extended base sequence or secondary structure specificity in the interaction of BP-diol epoxide with SV40 DNA.

Within the cell DNA is complexed with histones to form chromatin, and adduct formation may be affected by the relative accessibility

of the DNA. Preferential binding of BP-diol epoxide may occur with internucleosomal DNA as well as with DNA undergoing active replication and transcription (20). SV40 lytic infection in the presence of BP-diol epoxide would be a convenient in vivo system in which to study DNA alkylation.

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Footnotes

Abbreviations: BP, benzo[a]pyrene; BP-diol epoxide, ($\pm$) 7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; BP-tetraol, 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene; SV40, simian virus 40; FI DNA, covalently closed superhelical circles; A-T, adenine-thymine; G-C, guanine-cytosine; SDS, sodium dodecylsulfate; DMSO, dimethylsulfoxide; PFU, plaque forming unit.

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Figure Legends

- Figure 1. Concentration dependence of BP-diol epoxide (●---●) and BP-tetraol (■---■) binding to SV40 DNA.
- Figure 2. Distribution of BP-diol epoxide adducts among SV40 Hind III restriction fragments. The fragments are labeled A through F on the basis of decreasing size.
- Figure 3. Linear correlation of BP-diol epoxide binding with percent G-C content for SV40 Hind III restriction fragments.

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Fig. 1

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Fig. 2

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Fig. 3

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