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MASTER

THE MOLECULAR BIOLOGY OF ENVIRONMENTAL AROMATIC HYDROCARBONS

Progress Report

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Abstract

Studies on three different aspects of hydrocarbon action have been investigated. These studies concern: I) The biochemical effect of benzo(a)pyrene-diol-epoxide (BPDE) on the replication of Simian virus 40; II) The reaction of benzo(a)pyrene-7,8-diol (BPD) with viral DNA; and III) The effect of PAH derivatives on RNA transcription.

In project I, we have demonstrated the direct binding of BPDE to SV40 DNA in virus infected cells, which supports the observed inhibition of virus production by BPDE. Within 6-14 hours following BPDE treatment of SV40 infected cells, 50-60% of the viral DNA-BPDE adducts formed disappear, as compared to the amount of adduct found after 2 hours of treatment. Since viral DNA molecules in infected cells are quite stable, the disappearance of BPDE adducts in SV40 DNA suggests the possibility of specific adduct excision.

In project II, we have attempted to define the chemical nature of the complex formed between BPD and single-stranded DNA. Enzymatic digestion (DNase I, S1 nuclease, with or without alkaline phosphatase) of BPD bound to ØX174 DNA (either component being radioactive) was subjected to chromatography on paper and LH 20 sephadex. Chromatographic treatment of this type shows the presence of BPD complexes with either guanosine-containing nucleotides or nucleosides. Nevertheless, definitive proof of the chemical nature of such complexes has not been established. Preliminary examination indicates that the BPD-nucleoside complexes are not very stable, and may,

therefore, not be covalently linked.

In project III, two dimensional gel electrophoresis has been used for the fractionation of crude 4S ^{32}P-RNA prepared from rat liver following ^{32}Pi injection of rats. Autoradiography shows over 70 gel spots; 50 of these spots contain tRNA species as identified by amino acid charging. About half of the tRNA-containing spots represent single species while the remainder show charging with several amino acids. We expect to use this technique to determine if PAH administration to rats, or cells in culture, modify the production of individual tRNA species, qualitatively or quantitatively.

I. The effect of benzo(a) pyrene diolepoxide in the replication of Simian virus 40.

A. Background It is now well established that most activated chemical carcinogens bind covalently to nucleic acids. Reports from our laboratory have shown that carcinogenic polycylic aromatic hydrocarbons (PAH) inhibit the replication of both DNA- and RNA-containing bacterial viruses. Studies with anti-BPDE (trans -7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene) show that this metabolite of benzo(a)pyrene (BP) effectively alkylates bacteriophage ØX174 DNA and MS2 RNA, that it blocks replication of both viral genomes, and that a single alkylation event suffices to totally inactivate the capacity of these viral nucleic acids to induce new virus formation in *Escherichia coli*. Since the phage nucleic acids used in the above studies are single-stranded, it seemed important to determine whether double-stranded viral nucleic acids would respond in a similar manner to PAH exposure. We chose to examine the effect of BP derivatives on the replication of Simian virus 40 (SV40) because this virus contains duplex DNA of known sequence and replicates in animal cell lines (monkey kidney cells) rather than in bacterial cells.

In a recent report (G.T. Chang, R.G. Harvey, W-T. Hsu, and S.B. Weiss; Biochem. Biophys. Res. Commun. 88, 688, 1979) we observed that when SV40 infected cells were treated with anti-BPDE, virus production was partially inhibited. Similarly, when infectious SV40 DNA was alklated with anti-BPDE, its capacity to induce new virus formation was significantly reduced, however, single-hit

inactivation of SV40 DNA was not observed. We also found that if infected cells were pulsed with ^{3}H-thymidine shortly after BPDE treatment (2 hours), viral DNA synthesis was inhibited by about 85%. However, if SV40 infected cells were pulsed with labeled thymidine 24-36 hours after BPDE treatment, incorporation of radioactive thymidine into SV40 DNA was almost normal. These experiments indicate that shortly after the exposure of infected cells to BPDE, viral DNA synthesis is largely impaired, however, this impairment is reversible with time. One possible mechanism to explain these results would be that the treatment of virus infected cells with BPDE results in the formation of SV40 DNA-BPDE adducts which blocks viral DNA synthesis. These hydrocarbon-DNA adducts may be removed at longer incubation times and the damaged viral DNA molecules enzymatically repaired, thus allowing for the resumption of viral DNA synthesis.

B. Objectives for the current year

Our objectives for the current year have been to test the above hypothesis concerning the mechanism of action of BPDE inhibition of SV40 replication. Specifically, we wanted to answer the following questions.

1) Does the treatment of SV40 infected cells with anti-BPDE give rise to viral DNA-BPDE adducts?
2) If SV40 DNA-BPDE adducts are formed *in vivo*, what is the fate of these alkylated DNA molecules, i.e. do they undergo excision and repair?

C. Studies conducted in the current year and the results obtained.

1. Monkey kidney cells (CV-1) were infected with SV40 virus and after 48 hours of incubation treated with ^{3}H-BPDE for two hours. The infected cells were washed three times with media, harvested, lysed, and a "Hirt" supernatant prepared which contained most of the viral DNA. The supernatant was phenol extracted and nucleic acid precipitated with ethanol. The small precipitate was sedimented, dissolved in an appropriate buffer containing EDTA, and subjected to neutral and alkaline sucrose gradient centrifugation. Each centrifugation run contained ^{14}C-labeled SV40 DNA as marker. Following centrifugation, drops were collected from the bottom of the centrifuge tubes (40 fractions were collected) and assayed for ^{3}H and ^{14}C radioactivity in a Nuclear Chicago Mark III Scintillation Counter. For both neutral and alkaline centrifugations, tritium labeled material was found directly coincident with marker viral ^{14}C-DNA indicating that ^{3}H-BPDE treatment of SV40 infected cells results in the alkylation of viral DNA with the labeled hydrocarbon derivative.

2. In an attempt to determine the fate of alkylated viral DNA molecules, infected CV-1 cells were either exposed to ^{3}H-BPDE or pulse-labeled with ^{3}H-thymidine, chased, and then exposed to non-radioactive BPDE, during active viral DNA replication. Isolation of viral DNA 2 hours after ^{3}H-BPDE treatment showed that Form I SV40 DNA was complexed with the labeled hydrocarbon, however, at longer times of incubation (6 and 24 hours) the amount of

radioactivity in Form I DNA was less than half that found after 2 hours of BPDE exposure. SV40 DNA pre-labeled with ^{3}H-thymidine is quite stable in infected cells but when such cells were further exposed to BPDE (non-radioactive), disappearance of thymidine-labeled SV40 DNA was observed within 5-14 hours later. The above experiments strongly suggest that in infected cells, viral-DNA molecules alkylated with BPDE are specifically degraded or modified so that they no longer sediment as closed supertwisted circles (Form I). It is possible therefore, that the viral alkylated DNA molecules are targets for enzymatic excision-repair.

II. The reaction of benzo(a)pyrene-7,8-diol with nucleic acids.

A. Background

In a previous report from this laboratory (W.T. Hsu, D. Sagher, E.J. Lin, R.G. Harvey, P.P. Fu, and S.B. Weiss: Biochem. Biophys. Res. Commun., 87, 416, 1979) evidence was presented that certain dihydrodiol derivatives of benzo(a)pyrene and benz(a)anthracene, when incubated with ØX174 DNA *in vitro*, had the capacity to directly inactivate this infectious DNA. Binding studies with labeled trans-7,8-dihydrodiol-benzo(a)pyrene showed that this BP derivative formed complexes with various nucleic acids, *in-vitro*. Whereas diolepoxide derivatives of BP react equally well with both single- and double-stranded DNAs and RNAs, the BP-7,8-diol derivative formed complexes preferentially with single-stranded DNA and only minimally with RNA. In our preliminary studies, we also

found that the diol derivative reacted extensively with the deoxy-homopolymers of dI and dG while the diolepoxide reacted only with poly dG. These observations suggested that the diol and diolepoxide derivatives of BP recognize different binding sites in nucleic acids, and that PAH-diols may directly alter the expression of cellular macromolecules.

B. Objectives for the current year

Our objective for the current year has been to determine the chemical nature of the complex between BP-7,8-diol and nucleic acid.

C. Studies conducted in the current year

1. Radioactive ^{3}H-BP-7,8-diol (specific activity, 200mc per mM) was reacted with ØX174 DNA at room temperature for 10-15 min, the DNA precipitated with ethanol, washed with ethanol-acetone and re-suspended in an aqueous buffer solution. The DNA preparation was digested overnight with a mixture of DNase I, S1 nuclease, and alkaline phosphatase which resulted in the formation of the four deoxynucleosides and some undigested oligonucleotides of undetermined length. The digestion products were then chromatographed on a column of LH 20 sephadex and eluted with an increasing gradient of 30% to 100% methanol In this system free nucleosides and oligo-nucleotides elute early; nucleosides complexed with highly non-polar PAH derivatives elute at higher methanol concentrations. Several small peaks of radioactivity were observed in regions of the

gradient suggesting that they might be nucleoside-adducts of BP-diol, however, too little material was present to carry out further analysis. Also, a large portion of the radioactivity initially complexed with ØX174 DNA was lost and could not be accounted for. Subsequent attempts to provide larger quantities of diol-nucleoside complexes were unsuccessful largely because the amount of DNA-diol complex formed in the initial binding reaction appeared to be less and less with time. We suspect that the labeled BP-7,8-diol stored in tetrahydrofuran becomes inactivated due to reaction with peroxides. Another problem encountered was the loss of total radioactivity from the DNA-BP-diol complex following enzymatic digestion.

2. Fresh preparations of (^{3}H)BP-7,8-diol were obtained and incubated with ØX174 DNA. At the same time, ^{32}P-and (^{3}H)quanasine-labeled ØX174 DNA were reacted with non-labeled BP-7,8-diol. Following DNA isolation and washing, the reacted DNA was digested with an enzyme mixture containing S1 nuclease and DNase I to give 3'-nucleoside monophosphates, and then subjected to paper chromatography using various solvent systems. The results of these experiments indicated the presence of nucleotide-BP-diol complexes that migrate on paper chromatography differently from free nucleotides. Also, the primary BP-nucleotide complex appeared to contain GMP, however, this was not firmly established. When alkaline phosphatase was added to the above digestion mixture, chromatographic analysis

indicated that radioactivity was no longer associated with GMP but now migrated slightly faster than free guanosine but considerably slower than free BP-diol. These results suggest the presence of a complex between BP-diol and single-stranded ØX174 DNA which is not dissociated by enzymatic hydrolysis of the viral DNA but appears to result in nucleotide and/or nucleoside "adducts". Elucidation of the chemical nature of the BP-diol complex with DNA will require more work, however, preliminary analysis suggests that it may not involve covalent linkage.

III. The effect of PAH derivatives on RNA transcription.

A. Background

Studies in this and other laboratories have shown that the alkylation of DNA and RNA templates with activated PAH derivatives, *in vitro*, block the template function of these nucleic acids with respect to DNA, RNA, and protein synthesis. However, inhibition of nucleic acid and protein synthesis cannot readily account for the mutagenic effects also reported for activated hydrocarbons, which implies some change in the genetic code that is readily transmitted in dividing cells. Specific changes in the genetic code (nucleotide sequence arrangement) due to PAH administration to growing cells has not yet been described. If such changes do occur, one might expect that they would be random throughout the entire cell genome. Transfer RNAs are the most abundant class of DNA transcripts found in cells since they represent some 60 different RNA species, including isoacceptor tRNAs. If one could

examine the nucleotide sequence of all or most of the tRNA species following PAH administration, it is possible that specific base modifications might be observed thus establishing the capacity for PAH agents to modify the genetic code. Since conventional techniques for isolating a single tRNA species in pure form are tedious, and since there are so many different tRNA species, it seemed necessary to apply other techniques as a feasible approach to the above problem. Recent work in our laboratory using two-dimensional gel electrophoresis for isolating highly purified tRNA species suggests that this method might be applicable for the proposed study.

B. Objective

1. To isolate a large number of different animal cell tRNA species rapidly and of high purity, and to examine them for base modifications induced by PAH administration.

C. Work done in the current year.

We have been using two-dimensional (2D) gel electrophoresis for purifying T5 phage-coded tRNA species (reprint by C. Hunt et al., enclosed). This year, we have applied the 2D gel technique to crude rat liver 4S RNA, (labeled with ^{32}Pi by intraperitoneal administration) and have obtained a display of some 70 discrete radioactive gel spots following autoradiography. By extracting each gel spot and charging the recovered RNA with ^{3}H-labeled amino acids, we have located specific tRNA species

which recognize 15 different amino acids. We expect to complete the identification and gel location of the remaining tRNA species in the next few months. The work so far indicates that gel electrophoresis can be used for the rapid isolation of all the rat liver tRNA species in a highly purified form. The exact degree of purity for each separated species must await further analysis (i.e., reversed phase chromatography, fingerprinting, and possibly nucleotide sequence analysis). Studies with PAH on tRNA transcription are planned after the above work is completed.

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