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UVA Light-Induced DNA Cleavage by Selected Polycyclic Aromatic Hydrocarbons

S. Dong,[1] H.-M. Hwang,[2] C. Harrison,[1] L. Holloway,[1] X. Shi,[2] H. Yu[1]

[1] Department of Chemistry, Jackson State University, Jackson, MS 39217, USA
[2] Department of Biology, Jackson State University, Jackson, MS 39217, USA

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental carcinogens (Connell et al. 1997; Conney 1982; Dipple 1985; Lesko 1984). They are produced during forest tire, volcanic eruption, incomplete burning of fuel and other materials, tobacco smoke, and food processing (Baum 1978; Connell et al. 1997). Exposure to PAHs has been linked to the development of skin and lung cancers as it is summarized in the 8th Report on Carcinogens (National Toxicology Program, 1998). PAHs are considered relatively nontoxic themselves, but they can be activated after entering the cell. The first activation pathway is metabolism. PAH metabolic products, diol-epoxides or diones, have been known to be carcinogenic through DNA covalent adduct formation (Connell et al. 1997; Conney 1982; Devanesan et al. 1996; Dipple 1985; Lesko 1984). The diol-epoxides can alkylate DNA, usually by forming a bond to the exocyclic amino group of the guanine residue in duplex DNA (Geacintov et al. 1997). The diones are able to oxidatively damage DNA or form DNA covalent adducts through free radical intermediates (Chen et al. 1996; Devanesan et al. 1996).

Another pathway that enhances PAH toxicity is light activation. There have been studies on the photo-induced toxicity of individual PAH or mixtures toward organisms in the aquatic systems (Schirmer et al. 1999; Pelletier et al. 1997; Swartz et al. 1997). It is found that PAHs are generally more toxic when the system is exposed to the simulated solar radiation (ssr) than if it is kept in the dark. The increase in toxicity due to ssr may exceed 100 times (Swartz et al. 1997). It is suggested that PAHs act as photosensitizers (Pelletier et al. 1997). After absorbing UV light energy, PAHs in the excited-state may transfer their energy to molecular oxygen to produce reactive oxygen species that cause a variety of damages to the cell. The phototoxicity can also be due to the formation of DNA covalent adducts. Under light irradiation, benzo[a]pyrene can form DNA covalent adducts or cause DNA strand breakage (Blackburn et al. 1977; Brooks and Lawley 1964; Hoard et al. 1981; Santamaria et al. 1966; Striste et al. 1980). The presence of benzo[a]pyrene can also increase the formation of 8-hydroxy-2'-deoxyguanine (Liu et al. 1998) a compound generated by oxidative damage of DNA. It has been suggested that these DNA lesions are responsible for tumor induction and other adverse effects (Brooks and Lawley 1964; Camalier et al. 1981; Santamaria et al. 1966). However, the studies so far have mostly focused on benzo[a]pyrene alone. In this research, we examined light-induced DNA cleavage by some environmentally important 3, 4, 5-ring PAHs and their derivatives.

Correspondence to: H. Yu

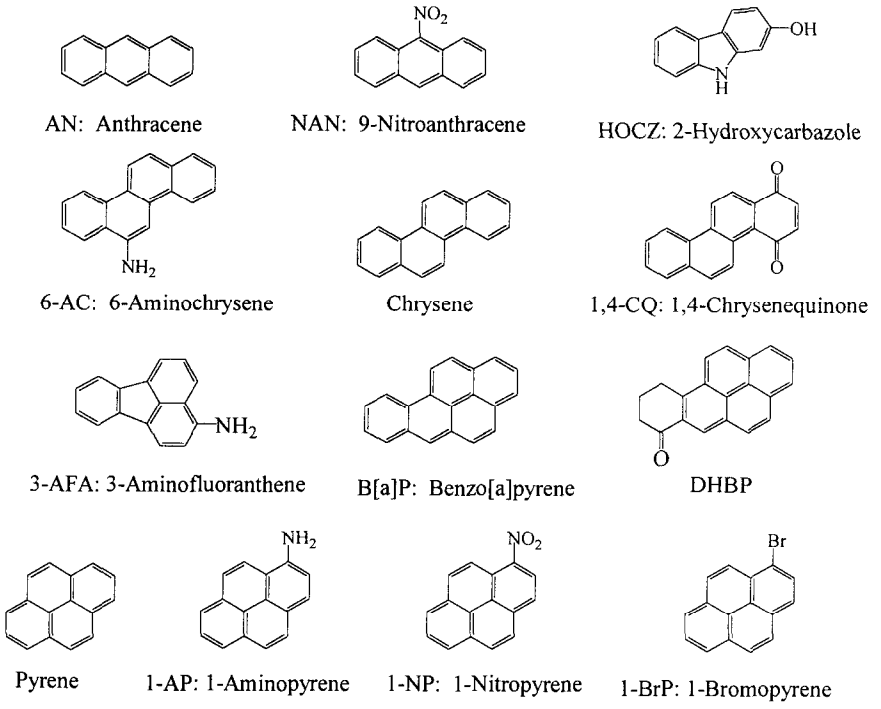

Figure 1. Structure and abbreviated names of PAHs used in this study

MATERIALS AND METHODS

Polycyclic Aromatic Hydrocarbons (Structures are listed in Figure 1) were purchased from Aldrich Chemical Co. (Milwaukee, WI). All of these compounds were in their highest purity grade and were used without further purification. PAH stock solutions, generally 1 mM, were prepared in dimethylformamide (DMF) and stored in brown containers in the refrigerator to exclude light. ΦX-174 Phage DNA (SO-90% supercoiled RF-l DNA with a molecular weight of 3.6×10^6 Da and approximately 5,386 base pairs) was purchased from Sigma Chemical (St. Louis, MO). Agarose, mono-, di-, and tri-basic sodium phosphate salt, TRIS base, boric acid, and EDTA were obtained from Fisher Scientific. All solvents used were spectroscopic grade. The water used (18 Ω) for buffer preparation was deionized by a Barnstead Nanopure Infinity water deionization system.

UVA-light induced plasmid DNA cleavage experiments were performed as following: Solutions (a total of 60 µL for each sample) containing ΦX174 Phage DNA and various amounts of PAHs were filled into the wells of a 3x8 flat-bottomed Titertek™ plate (ICN Biochemicals). The DNA concentration was set at 27 µM in base pairs in all experiments. The solution was buffered with 10 mM sodium phosphate at pH 7.1 and contained 4% DMF as co-solvent for better PAH solubility. Other solvent systems used in this study were 10% methanol and 20% DMF. The Titertek plate was tightly covered with a piece of glass plate and placed onto a Pyrex glass support/filter, which was placed

on an O-ring secured on a ring stand. The Pyrex glass served as a light filter to efficiently cut off any light below the wavelength of 300 nm that would potentially damage DNA. The absorbance of the Pyrex glass was checked on the Varian CARY 300 UV-Vis spectrophotometer. Over 98% of light below the wavelength of 300 nm was filtered off. A 100 W UV lamp (type B, UVP Inc., Upland, CA) was placed beneath the Pyrex glass and the light was applied through the bottom of the Titertek plate from a fixed distance of 6.5 cm. The intensity of the light output was measured to be 170 J/cm^2 per hour (UVA detector, Model PMA 2100, Solar Light Co., Inc., Philadelphia, PA). A stream of cold air blowing through the bottom of the Pyrex glass was used to eliminate any heat generated by the light source.

After irradiation for one hour, 10 µL of a gel-loading dye solution (bromophenol blue and xylencyanol in 50% glycerol) was added into each well. Then 10 µL of the sample was loaded into the wells of a 1% agarose gel. The gel was run in 1× TBE buffer (pH=8.27) at 105 volts for 70-90 min at room temperature. Following electrophoresis, the gel was stained with ethidium bromide (2 mg/L) for 30 min and analyzed with a NucleoVision Gel-Documentation System (NucleoTech Inc., CA). The amount of the supercoiled Form I DNA (SC-DNA) and the relaxed open circular Form II DNA (oc-DNA) were quantified by the total fluorescence intensity of the bands after subtracting a common background. A coefficient of 1.66 was used to correct the lower efficiency of ethidium bromide binding to the SC-DNA than to the oc-DNA (Ciulla et al. 1989). The fraction of SC-DNA, X_{sc}, was calculated by equation 1,

$$X_{sc} = A_{sc}/(A_{sc} + A_{oc}/1.66) \quad (1)$$

where A_{sc} and A_{oc} are the total fluorescence intensities for the SC-DNA and oc-DNA bands, respectively. Percent of SC-DNA cleavage or percent of SC-DNA converted into oc-DNA is calculated by equation 2,

$$\% \text{ sc-DNA cleavage} = (1 - X_{sc} / X_{sc}^{o}) \times 100\% \quad (2)$$

where X_{sc}^{o} is the initial percentage of the SC-DNA before cleavage. Both the X_{sc} and X_{sc}^{o} are calculated from equation 1. A plot of the percent of SC-DNA cleavage versus PAH concentration was used to determine C_{25}, the PAH concentration that causes 25% sc-DNA cleavage. The C_{25} values were used for comparing the potency of each PAH in terms of causing light-induced DNA cleavage. Preliminary experiments using a much wider concentration range were first conducted to obtain an approximate C_{25} value. A further experiment in the concentration range near the approximate C_{25} was carried out to determine a more accurate C_{25} value. The reason to choose C_{25}, instead of the more traditional C_{50}, is that the SC-DNA needs only a single cut (single strand break) to be converted into oc-DNA. As the concentration of the oc-DNA increases due to this sc-DNA to oc-DNA conversion, oc-DNA can also be cut by PAH. Therefore, significant errors will occur in determining the effective concentration of the PAH that only cuts the SC-DNA.

Time course of light-induced ΦX-174 phage DNA cleavage was carried out in a quartz cuvette containing DNA/PAH mixtures. The cuvette was placed on top of the Pyrex glass support/filter. The light source was set the same way as described above. Samples were taken after a period of irradiation and stored immediately in the dark. After all

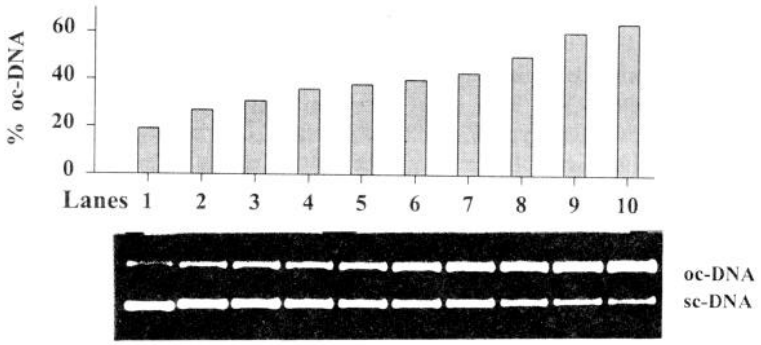

Figure 2. Irradiation time dependence of UVA-induced DNA cleavage by 1 -AP. The ΦX- 174 DNA and 1 -AP concentrations were 27 μM and 6 μM, respectively, in all samples. Lane 1 is the control sample before irradiaion. Lanes 2-10 were samples taken after 2, 4, 6, 10, 15, 20, 30, 40, 60 min of irradiation by a 100 W UVA lamp.

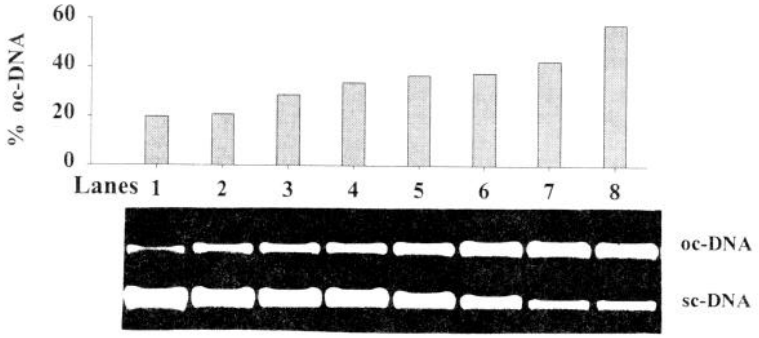

Figure 3. 1 -AP concentration dependence of DNA cleavage. The irradiation time was one hour using a 100 W UVA lamp. The ΦX-l74 DNA concentration was 27 μM in all samples. Controls: lane 1: 8 μM 1-AP + DNA, no light; lane 2: DNA alone under light. Lanes 3-8 were with 1, 2, 3, 4, 6, 8 μM 1-AP and light.

samples were taken, they were treated and subjected to electrophoresis and quantified as described above.

RESULTS AND DISCUSSION

UVA-light induced DNA cleavage by PAHs is both PAH concentration and irradiation time dependent. Figure 2 shows the UVA dose dependence of the DNA cleavage induced by 6 μM 1 -AP. The bottom bands represent the SC-DNA, and the top bands represent the oc-DNA. The bar graph above the gel picture refers to the quantified band intensity for the oc-DNA. Lane 1 is the sample taken just before irradiation and it represents the original constitution of the DNA with 80% SC-DNA and 20% oc-DNA. As irradiation time increases, the amount of SC-DNA decreases and the amount of the oc-DNA increases, signifying that the SC-DNA was converted to the oc-DNA. This conversion is due to DNA single strand cleavage caused by 1-AP and light. It is known that a knick or

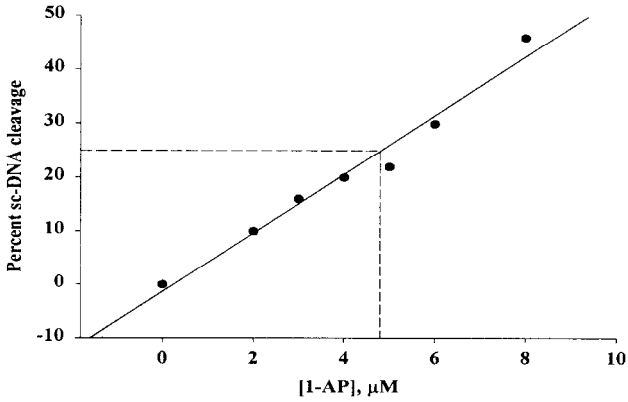

Figure 4. Plot of percent of SC-DNA cleavage versus 1-AP concentration for (C_{25} determination. The C_{25} is determined from the graph as shown.

a single strand cleavage of the SC-DNA converts it to the oc-DNA. The absence of a band for the linear form-III DNA suggests that the DNA cleavage is single, not double stranded. Otherwise, the linear DNA band would have appeared between the SC-DNA and the oc-DNA bands. Other PAHs tested also showed similar irradiation time dependence.

The PAH concentration-dependence of DNA cleavage was tested by mixing the phage DNA with various amounts of PAHs in the buffer with 4% DMF. Figure 3 is the picture of the gel for the ΦX- 174 Phage DNA treated with various concentrations of 1 -AP after 1 hr of irradiation. The bands representing the oc-DNA were quantified and shown as a bar graph above the picture of the gel. Control experiments show that the percent of SC-DNA remains about the same as the original DNA's 80%, whether the DNA was or kept in the dark in the presence of 8 μM 1-AP (lane 1) or irradiated by light in the absence of 1-AP (lane 2). As the concentration of added 1-AP increases from 1-8 μM (lanes 3-8) the amount of SC-DNA decreases while the amount of oc-DNA increases due to single stranded cleavages caused by 1-AP and light. All other PAHs tested also exhibited the same concentration dependence.

The relative DNA photocleavage efficiency expressed as C_{25}, the PAH concentration at which 25% of the original SC-DNA was converted into oc-DNA, was determined by plotting the percent of SC-DNA cleavage versus the PAH concentration as shown in Figure 4 for 1-AP. All PAHs examined were able to cause light-induced DNA cleavage and generate a linear graph as shown for 1-AP. The C_{25} was obtained directly from the graph by determining the concentration of the PAH that corresponds to a 25% sc-DNA cleavage. Table 1 lists the C_{25} data for all PAHs examined in three solvent systems: all with 10 mM phosphate buffers, but with 4% DMF, 10% MeOH and 20% DMF, respectively. The "n. d." for 1-NP in 20% DMF means there is no linear relationship between the percent of SC-DNA cleavage and 1-NP concentration.

Table 1. Relative DNA photocleavage potency of PAHs expressed by C_{25} (μM)

PAHs	4% DMF	10% MeOH	20% DMF
Benzo[a]pyrene (B[a]P)	6.0		4.9
Pyrene	51		
1-Aminopyrene (1-AP)	4.2	1.9	4.4
1-Hydroxypyrene (1-HOP)	0.36	0.38	4.8
1-Nitropyrene (1-NP)	9.9		n. d.
1-Bromopyrene (1-Br-P)	4.0		11
9,10-Dihydrobenzo[a]pyren-7(8*H*)-one (DHBP)	15		19
Chrysene	5.2	4.1	
6-Aminochrysene (6-AC)	60	4.2	
1,4-Chrysenequinone (1,4-CQ)	5.8	10	
3-Aminofluoranthene (3-AFA)	32	4.8	
Anthracene (AN)	8.3		
9-Nitro-anthracene (9-NAN)	0.83		
2-Hydroxycarbazole (HOCZ)	> 100	57	

By comparing the C_{25} values for the PAHs obtained in different solvents, it is obvious that solvents could have a profound effect on the light-induced DNA cleavage by some PAHs. For example, the C_{25} values in 4% DMF for the amino substituted PAHs, 1-AP, 3-AFA, and 6-AC are 2-14 folds lower than when they are measured in 10% methanol. The C_{25} for l-HOP obtained in 4% DMF or 10% MeOH is 13 folds higher than when it is obtained in 20% DMF. Also, the solvent effect on the C_{25} values of individual PAHs is different. While the C_{25} value is greatly changed for the amino substituted PAHs going from 4% DMF to 10% methanol as solvents, the C_{25} values for 1 -HOP, chrysene and 1,4-CQ are only slightly affected due to the solvent change. However, while the C_{25} value is increased by 13 folds for l-HOP going from 4% DMF or 10% MeOH to 20% DMF, it only increases slightly for other PAHs. This difference in solvent effects on the light-induced DNA cleavage efficiency might be due to different mechanisms leading to DNA cleavage. Mechanistic studies on the light-induced DNA cleavage by some PAHs will be published elsewhere (Dong et el. unpublished results).

When all PAHs are compared within a solvent system (4% DMF), l-HOP and 9-NA are the two most potent compounds in terms of causing light-induced DNA cleavage, with C_{25} values of 0.36 and 0.83 μM, respectively. Eight of the 14 tested PAHs belong to the next group of compounds that have C_{25} values of 4.0-15 μM, one order of magnitude higher than l-HOP and 9-NA. The C_{25} values for pyrene, 6-AC, and 3-AFA are another order of magnitude higher, while the C_{25} for HOCZ is too high to be determined.

Substituents of PAHs have a strong effect on the UVA light-induced DNA cleavage, too. In comparison to AN, its nitro substituted derivative 9-NAN has a ten fold lower C_{25}. Substitution with a nitro, amino, or bromo group on pyrene lowers the C_{25} by one order of magnitude, while the hydroxy substitution lowers the C_{25} by two orders of magnitude. In contrast, an amino substitution on chrysene increases the C_{25} by one order of magnitude. We believe that the effect of a substitution group of a PAH on the DNA cleavage potency of the compound depends on at least two properties: The UV absorption spectra of the individual PAH and the mechanism that causes DNA cleavage.

It is suggested that the phototoxicity of PAHs be due to light-induced generation of reactive oxygen species that damage biomolecules (Newsted and Giesy 1987; Pelletier et al. 1997; Swartz et al. 1997). Here we identified one of the possible damages to be light-induced DNA single stranded cleavage. All 14 compounds tested are able to cause a certain amount of DNA cleavage, although their cleavage potency is quite different. Also, since the damage is to DNA, it is possible that this lesion is genotoxic, in addition to being acutely toxic. However, acute toxicity and genotoxicity data for these PAHs are needed to understand whether the phototoxicity of these compounds relates to their light-induced DNA cleavage. While there are some phototoxicity data for the non-substituted PAHs (Pelletier et al. 1997; Swartz et al. 1997; Takeda et al. 1984) there are very little data available for the phototoxicity of the substituted PAHs.

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