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Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list

Jian Yan^a, Lei Wang^a, Peter P. Fu^b, and Hongtao Yu^{a,*}

^aDepartment of Chemistry, Jackson State University, Jackson, MS 39217, USA

^bNational Center for Toxicological Research, Jefferson, AR 72079, USA

Abstract

The photomutagenicity of 16 polycyclic aromatic hydrocarbons (PAHs), all on the United States Environmental Protection Agency (US EPA) priority pollutant list, was studied. Concomitant exposing the *Salmonella typhimurium* bacteria strain TA102 to one of the PAHs and light (1.1 J/cm² UVA+2.1 J/cm² visible) without the activation enzyme S9, strong photomutagenic response is observed for anthracene, benz[*a*]anthracene, benzo[*ghi*]perylene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and pyrene. Under the same conditions, acenaphthene, acenaphthylene, benzo[*k*]fluoranthene, chrysene, and fluorene are weakly photomutagenic. Benzo[*b*]fluoranthene, fluoranthene, naphthalene, phenanthrene, and dibenz[*a,h*]anthracene are not photomutagenic. These results indicate that PAHs can be activated by light and become mutagenic in *Salmonella* TA102 bacteria. At the same time, the mutagenicity for all the 16 PAHs was examined with the standard mutagenicity test with 10% S9 as the activation system. Benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, acenaphthylene, and fluorene are weakly mutagenic, while the rest of the PAHs are not. In general, the photomutagenicity of PAHs in TA102 does not correlate with their S9-activated mutagenicity in either TA102 or TA98/TA100 since they involve different activation mechanisms.

Keywords

Polycyclic aromatic hydrocarbons; Photomutagenicity; Light irradiation; *Salmonella typhimurium* TA102 and TA98

1. Introduction

Sunlight is a complete carcinogen and is responsible for the induction of squamous cell and basal cell carcinomas in humans [1–7]. The solar radiation is composed of 91% visible (400–700 nm), 8.7% UVA (320–400 nm), and 0.3% UVB (280–320 nm) light [8]. Although UVB is only a small fraction of the overall solar radiation, it is the main cancer causing component of the solar light [9]. UVA is much less harmful than UVB. Exposure to UVA can cause sunburn and it is also believed that UVA is weakly carcinogenic [10]. Visible light is generally regarded as not carcinogenic or mutagenic.

Combustion of fossil fuels is always incomplete and generates polycyclic aromatic hydrocarbons (PAHs) as a class of most spread mutagenic and carcinogenic particulate environmental pollutants. The United States Environmental Protection Agency's (US EPA)

Toxic Release Inventory estimated that more than 5 million pounds of PAHs are among the toxicants released in the year 2000 [11]. Human contamination with PAHs is inevitable through mainly skin absorption, inhalation, or food consumption. PAH contaminated skin may be exposed to sunlight irradiation. It has been suggested that concomitant exposure to chemicals and light can cause toxic reactions and this is termed phototoxicity [12–15]. Because of the multiple aromatic ring systems in PAHs, these compounds can absorb light energy in the UVA and for some in the visible region [16], forming reactive species and causing damages to human cellular components. It has been shown that concomitant exposure to PAHs and light can cause DNA single strand cleavage, oxidation of DNA bases, and form DNA covalent adducts [17–21]. In this article, we report the photomutagenicity of all the 16 PAHs listed in the US EPA priority pollutant list [22] in *Salmonella typhimurium* TA102 concomitantly under light (UVA + visible) irradiation. The structures and nomenclatures of these 16 PAHs are shown in Fig. 1.

2. Material and methods

2.1. Materials

S. typhimurium TA102 and TA98 strains were kindly provided by Dr. Bruce Ames from the University of California (Berkeley, CA). The 16 PAHs selected for this study are on the US EPA list of 126 priority pollutants [22]. Fourteen of the 16 PAHs, acenaphthene, acenaphthylene, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene, along with the positive control compound 8-methoxypsoralen (8-MOP), were purchased from Sigma–Aldrich (St. Louis, MO). Benzo[*b*]fluoranthene and indeno[1,2,3-*cd*]pyrene were from Ultra Scientific (North Kingstown, RI). Other chemicals and solvents were used in their highest purity grade. Aroclor 1254 induced S9 (35 mg/ml of protein) was purchased from ICN biochemicals (Aurora, OH).

2.2. Light source

The light source used in this study was a 300 W Xe/Hg(Xe) lamp from ORIEL Instruments (Stratford, CT), producing a full-spectrum light ranging from 300 to 800 nm. A Pyrex glass plate (1 mm in thickness) was placed 40 cm above the light bulb. The light output energy is 3.8 mW/cm² for UVA and 7.0 mW/cm² for visible light measured by a photo-radiometer from Solar Light Co. (Philadelphia, PA) equipped with UVA, UVB, and visible light probes. Comparing with the natural sunlight [8], the light for this study has a higher UVA content. UVB is mostly filtered off by the Pyrex glass with measured output UVB energy of 0.012 mW/cm². The samples were irradiated either for 5 or 30 min. The light doses measured after passing through the Pyrex glass filter and the Petri dish cover are: (1) 5 min irradiation: 1.1 J/cm² for UVA and 2.1 J/cm² for visible light or a combined light dose of 3.3 J/cm²; and (2) 30 min irradiation: 6.6 J/cm² for UVA and 12.6 J/cm² for visible light or a combined light dose of 19.8 J/cm².

2.3. Photomutagenicity tests

The assays were conducted in *S. typhimurium* histidine auxotrophic strain TA102 concomitant with irradiation by light in the presence of a PAH as described previously [23], which follows the method of Maron and Ames [24], a standardized procedure for assays in the absence of light irradiation, and the experimental conditions of Utesch and Splittgerber modified for photomutagenicity tests [25]. The positive control of photomutagenicity was the commonly used 8-MOP [24–26]. Briefly, 1 ml of a freshly prepared solution of a test PAH in dimethylsulfoxide was placed in a 13 mm × 100 mm capped culture tube, to which was added with 1 ml of overnight TA102 culture and 5 ml of sodium phosphate buffer (20 mM, pH 7.4). After mixing, it was incubated at 37 °C for 20 min at 210 rpm with a loose fitting cap. In a beaker, melted top agar (100 ml) was added with 10 ml histidine/biotin solution (both 0.5 mM)

and mixed thoroughly. Two milliliters of the top agar/histidine/biotin mixture was transferred into each of the 13mm × 100mm capped culture tubes at 45 °C in a heating block, followed by addition of 0.7 ml pre-incubated PAH-TA102 culture solution. The combined 2.7 ml mixture was poured onto minimal glucose agar plates with the cover on. The plates were left on a leveled surface to allow the agar to harden before placed on top of the Pyrex glass support upside down. Light was allowed to irradiate from the bottom up through both the Pyrex glass and the culture plate cover for 5 or 30min. After irradiation, the plates were incubated for 48 h at 37 °C and the revertant colonies were counted with a colony counter.

Control experiments on the effect of light irradiation on both TA98 and TA102 were conducted and the responses are shown in Fig. 2 and Fig. 3. Most of the strain TA98 bacteria died after exposing to a light dose of 0.6 J/cm² only (1 min of irradiation) because TA98 lacks the DNA repair capability. On the contrary, TA102 could survive up to a light dose of 19.0 J/cm² (30 min of irradiation) because it has the DNA repair system intact [24]. Therefore, TA102 is the only strain used for this preliminary study on the photomutagenicity of this series of PAHs. Fig. 4 is the positive control experimental results for 8-MOP on TA102 upon light irradiation. The number of revertant colonies reaches 2500 per plate after a light dose of 1.9 J/cm².

All compounds were assayed in at least two separate occasions and obtained similar results. The variation in revertants per plate between replicate assays was generally <20%. The number of revertants induced per nanomole of a PAH was calculated from the linear regression analysis of the increasing portion of the dose–response curve. A negative solvent control (no light), a light control (without PAH), and an 8-MOP positive control were used throughout all experiments. The average numbers of revertant colonies per plate for 5 min of light irradiation control (without PAH) was 548 ± 71, 1.5–1.9 times of the solvent negative control (no light). This indicates that the light used in this study is slightly mutagenic.

2.4. S9-activated mutagenicity of PAHs in TA102

There is no experimental data on S9-mediated mutagenicity of PAHs in TA102 except for benzo[a]pyrene [27]. In our study, the standard mutagenicity test with 10% S9 was used which followed the method of Maron and Ames [24], where benzo[a]pyrene is usually used as a positive control. Benzo[a]pyrene, 1 or 15 µg per plate, and all the other 15 PAHs were examined with the same concentrations of PAHs as the photomutagenicity test. All experiments were repeated at least once. Negative control without PAH, or control with PAH but without S9 mix, and benzo[a]pyrene positive control were conducted with each set of experiments. The average numbers of revertant colonies per plate for S9 mix control without PAHs were 472 ± 61, 1.4 times of the control without S9. The average numbers of revertant colonies per plate for 1.0 or 15 µg per plate of benzo[a]pyrene positive controls with S9-mix were 539 ± 61 or 920 ± 72, 1.6 or 2.8 times the control without S9.

3. Results

A total of 16 PAHs (Fig. 1) on the EPA priority pollutant list were selected for photomutagenicity tests in *Salmonella* TA102 concomitantly with UVA and visible light irradiation. Following the above described experimental procedures, the photomutagenicity of each of the 16 PAHs in the doses of 0, 13.5, 67.5, 337.5, 1687.5 nanomole per plate (or concentrations of 0, 5, 25, 125, and 625 µM) was initially attempted. However, precipitation was observed with a dose of 337.5 nanomole per plate or higher due to low solubility of PAHs in aqueous solutions [28]. Besides, benz[a]anthracene, benzo[a]pyrene, anthracene, benzo[ghi]perylene, indeno[1,2,3-*cd*]pyrene, and pyrene at 13.5 nanomole per plate are highly photocytotoxic, leading to bacteria death. Therefore, lower doses of 2.7 nanomoles or less were used for further tests of these PAHs.

The number of revertant colonies per plate caused by concomitant exposure to light and pyrene or anthracene, as examples for all the 16 PAHs tested, is plotted versus their concentrations in Fig. 5. For pyrene at the lowest dose, 0.14 nanomoles per plate, the number of revertant colonies is about two times the control (light irradiation in the absence of PAH), signifying that pyrene is photomutagenic. At this and the next two doses (0.27 and 0.54 nanomoles per plate), the number of revertant colonies remains about the same before it decreases at the higher doses (1.1 and 2.7 nanomoles per plate). This is a sign for cytotoxicity causing bacteria death. At the pyrene dose of 13.5 nanomoles per plate, nearly all bacteria are dead. For anthracene, there is no increase in the number of revertant colonies at the anthracene dose of 0.054 nanomoles per plate comparing with the control, but the number of revertant colonies increases to about twice the control at 0.11 nanomoles per plate and continues to increase till 0.54 nanomoles per plate before leveling off.

The photomutagenicity of the other 14 PAHs was similarly determined as those described for pyrene and anthracene in Fig. 5. Based on the data points in the increasing region of the dose–response curve, the number of revertant colonies per plate and per nanomole of a PAH are obtained and listed in Table 1. A positive photomutagenicity (+) is scored if the number of revertant colonies per plate due to exposure to light and a PAH exceeds twice the light control. A strong photomutagenicity (++) is scored if the number of revertant colonies per nanomole of a PAH is higher than 2000. All the PAHs were also treated with a greater light dose (30 min of irradiation) at a selected concentration to test the effect of light dose on the photomutagenicity. The data are also listed in Table 1 and are used to confirm the photomutagenicity for those PAHs that are borderline photomutagenic with 5 min of irradiation. Under 30 min of irradiation, most PAHs either become stronger photomutagenically or caused bacteria death (6 PAHs in Table 1).

As shown in Table 1, 11 of the 16 PAHs tested are photomutagenic. Based on the number of revertants per nanomole of a PAH, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, and pyrene are identified as strongly photomutagenic with the number of revertant colonies per nanomole of the PAH in the range of 2000–20,000. They also caused bacteria death either at 13.5 nanomoles per plate for the 5 min light irradiation or the listed dose at 30 min of light irradiation (Table 1). Acenaphthene, acenaphthylene, benzo[*k*]fluoranthene, chrysene, and fluorene are weakly photomutagenic. Longer irradiation time enabled benzo[*k*]fluoranthene, chrysene, and fluorene to cause significantly more revertant colonies. The rest of the five PAHs also caused a higher number of revertant bacteria colonies (20–50% higher than the light control) due to concomitant exposure to a PAH and light irradiation comparing with the light control, but they are not high enough to be scored as a positive photomutagenic compound.

These 16 PAHs were evaluated with S9 mix as external metabolizing enzyme system. There was no mutagenicity without S9 mix for all 16 PAHs. Benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and chrysene, along with benzo[*a*]pyrene, showed that the number of revertant colonies due to the exposure to the PAH and S9 is about 1.5 times the control with S9 mix and >2 times than the solvent negative control without S9. Fig. 6 is an example for benzo[*k*]fluoranthene. The rest of the PAHs showed little or no mutagenic response for tested doses.

4. Discussion

There are several reports on photomutagenicity test of chemicals [12,23,25,26,34–36] and there are no guidelines set for photomutagenicity tests in terms of the number of bacteria strains and light doses as determined by a working group of scientists on photochemical genotoxicity [37]. In this paper, we report the preliminary photomutagenicity test on the 16 PAHs listed as US EPA priority toxic pollutants using *S. typhimurium* TA102. Under our experimental

conditions, six PAHs are highly photomutagenic, five are weakly photomutagenic, and five are not photomutagenic (Table 1). The six highly photomutagenic compounds are anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, and pyrene with the amounts of each PAH used for the tests being 0.1, 0.1, 0.01, 0.03, 0.07, and 0.03 μg per plate, respectively. Under these low dose conditions, there were weak mutagenicity of 3 PAHs with S9 as the external metabolizing system. In comparison to the doses used for S9-activated systems in TA98/TA100, the doses for the same PAH needed to cause S9-activated mutagenicity is in the range of 1–1000 μg per plate [32,33]. This amount is 2–5 orders of magnitude higher than the amount used in this photomutagenicity test. This might indicate that it requires much less PAHs to cause photomutagenicity than enzyme activated mutagenicity, although the efficiency for the S9-activated reaction or light-activated reaction must also be considered.

In comparison to the S9-activated mutagenicity data or carcinogenicity data on experimental rodents, some of the mutagenic and/or carcinogenic PAHs are not photomutagenic and some of the non-mutagenic and/or non-carcinogenic PAHs are photomutagenic (Table 1). Benzo[*b*]fluoranthene, dibenz[*a,h*]anthracene, and fluoranthene are not photomutagenic, although they are both mutagenic upon S9-activation and carcinogenic in experimental rodents (except for fluoranthene). On the other hand, the six non-mutagenic and non-carcinogenic PAHs, acenaphthene, acenaphthylene, anthracene benzo[*ghi*]perylene, fluorene, and pyrene, are photomutagenic (there is no sufficient carcinogenicity data on experimental rodents for acenaphthylene, benzo[*ghi*]perylene, and fluorene). Only benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, chrysene, and indeno[1,2,3-*cd*]pyrene are both photomutagenic and mutagenic with S9 activation. Furthermore, for comparison purposes, all 16 PAHs were evaluated with S9 mix under the same chemical doses as photomutagenicity experiments, where only benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and chrysene were mutagenic.

It is worth noting that some of the PAHs are phototoxic or photocytotoxic toward the *Salmonella* bacteria. Among the 16 PAHs tested, bacteria death was observed due to concomitant exposure to light and anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, or pyrene. Coincidentally, these compounds are also the strong photomutagenic PAHs.

Phototoxicity, including photomutagenicity, is closely related to the photochemical reactions in terms of generation of reactive PAH intermediates and reactive oxygen species during photolysis [17]. Depending on the PAH species and the location of the substituent groups, the reactive oxygen species or PAH intermediates generated due to light activation can be different [17,20,38,39]. The photochemical reaction rate, reaction pathways, and effect of solvent and co-existing chemicals/ions on the photochemical reaction rate or pathway for individual PAHs are different and these factors can also influence photo-toxicity. Light-induced DNA cleavage or DNA adduct formation is greatly affected by co-existing chemicals [18,20,38,39]. Likewise, the photodegradation rate and the percent of each photoproduct are greatly affected by various factors [40–43]. Therefore, detailed mechanistic understanding on the photochemistry of PAHs is needed in order to determine the relationship between photomutagenicity and structure of the PAHs.

Since PAHs are ubiquitous in the environment and concomitant exposure to PAHs and light by humans is inevitable, photomutagenicity of these compounds are of human health importance. It has been well established that PAHs require metabolic activation to exert their biological activities, including mutagenicity or carcinogenicity, mediated through formation of reactive metabolites that can cause DNA damages or form DNA covalent adducts [44–50]. The photomutagenicity of PAHs reported here indicates that there may be a different route leading to adverse human health effects, e.g., PAHs can be activated by light irradiation,

without requiring metabolizing enzymes. Especially, there are six PAHs among the 16 tested, including those that are most abundant in the environment, acenaphthene, acenaphthylene, anthracene, benzo[ghi]perylene, fluorene, and pyrene, that are not mutagenic in cells through metabolic activation, but are photomutagenic in TA102. This result may affect the way PAH risk assessment is conducted. Although studies for the understanding of the mutagenicity and carcinogenicity of PAHs upon metabolic activation are very important, possible adverse health effects due to skin contamination with PAHs and exposure to sunlight irradiation at the same time should not be overlooked.

Abbreviations

PAHs, polycyclic aromatic hydrocarbons; 8-MOP, 8-methoxypsoralen; US EPA, United States Environmental Protection Agency; UVA, 320–400 nm; UVB, 280–320 nm.

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Fig. 1.
Structures and nomenclatures of the 16 PAHs on the EPA priority pollutant list.

Fig. 2.
Effect of light doses from the 300 W Xe/Hg lamp on *S. typhimurium* TA98.

Fig. 3.
Effect of light doses from the 300 W Xe/Hg lamp on *S. typhimurium* TA102.

Fig. 4.
Photomutagenic response of *S. typhimurium* TA102 upon exposure to 10 μ g per plate of 8-MOP and various light doses from the 300 W Xe/Hg lamp.

Fig. 5.

Photomutagenicity of pyrene and anthracene in *Salmonella typhimurium* TA102. The PAH assayed at different amount of chemicals was mixed with TA102 in the agar plate and irradiated with a Xe/Hg lamp for 5 min (light dose: 1.1 J/cm² UVA plus 2.1 J/cm² visible light). After the irradiation, it was incubated for 48 h before the revertant colonies were counted.

Fig. 6. Mutagenicity of benzo[k]fluoranthene in *Salmonella typhimurium* TA102. Values are means of three parallel incubations without S9 (○) and with S9 mix as an external metabolizing system (●).

Table 1

Comparison of photomutagenicity of the 16 PAHs on the list of US EPA 126 priority pollutants with their respective carcinogenicity and mutagenicity reported in the literature

PAH name (EPA # ^d) CAS #	Revertants/nmol (5 min)	Photomutagenicity ^b		Carcinogenicity ^c	Mutagenicity ^d
		Revertants per plate (μg) (5 min)	Revertants per plate (μg) (30 min)		
Acenaphthene (1) 83-32-9	+	0.6 ± 0.2	1018 ± 78 (265)	0	- ^e
Acenaphthylene (77) 208-96-8	+	17 ± 1	1178 ± 57 (10)	I	- ^e
Anthracene (78) 120-12-7	++	2222 ± 78	Bacteria death (2.4)	0	-
Benz[<i>a</i>]anthracene (72) 56-55-3	++	2037 ± 167	Bacteria death (3.0)	++	+
Benzo[<i>a</i>]pyrene (73) 50-32-8	++	19185 ± 648	Bacteria death (3.4)	++	+
Benzo[<i>b</i>]fluoranthene (74) 205-99-2	-	63 ± 11	739 ± 211 (3.4)	++	+ ^e
Benzo[<i>ghi</i>]perylene (79) 191-24-2	++	11287 ± 917	Bacteria death (3.7)	I	- ^e
Benzo[<i>k</i>]fluoranthene (75) 207-08-9	+	69 ± 10	2535 ± 357 (3.4)	++	+ ^e
Chrysene (76) 218-01-9	+	346 ± 44	1733 ± 96 (3)	+	+
Dibenz[<i>a,h</i>]anthracene (82) 53-70-3	-	13 ± 0.1	729 ± 63 (3.7)	++	+
Fluoranthene (39) 206-44-0	-	11 ± 0.6	706 ± 179 (2.7)	0	+ ^e
Fluorene (80) 86-73-7	+	0.6 ± 0.1	1858 ± 180 (2.2)	I	-
Indeno[1,2,3- <i>cd</i>]pyrene(83) 193-39-5	++	4652 ± 178	Bacteria death (0.07)	++ ^f	+ ^c
Naphthalene (55) 91-20-3	-	0.6 ± 0.1	665 ± 147 (864)	I	-
Phenanthrene (81) 85-01-8	-	3.2 ± 0.3	820 ± 79 (300)	I	-
Pyrene (84) 129-00-0	++	8926 ± 311	Bacteria death (2.7)	0	-

^aThe numbers in parenthesis are the numbers from the EPA list of priority pollutants [22].

^bAll experiments were conducted with *S. typhimurium* TA102 with light irradiation. The assays were performed in triplicates and the data were reported from linear regression analysis of the increasing region of the dose-response curve without subtracting the number of revertants for the light control which was 548 ± 71 per plate. A PAH is defined as photomutagenic (+ or ++) when the number of revertant colonies due to concomitant exposure to light and the PAH is greater than twice of the light control. A PAH is defined as strong photomutagenic (++) if its revertant colonies per nanomole of the PAH is more than 2000. Bacteria death means that more than 90% of the bacteria died based on viable bacteria count at the doses given in the bracket.

^cData summarized from IARC report [29], NTP report [30], White [31], and from the EPA web site for acenaphthylene, naphthalene, and fluorene. Carcinogenicity symbols are: (0) no evidence of carcinogenicity; (I) inadequate evidence for evaluation; (+) limited evidence of carcinogenicity in experimental animals; (++) sufficient evidence of carcinogenicity in experimental animals.

^dBased on report by McCann et al. [32] using *S. typhimurium* TA98 or TA100 with S9 activation.

^eNagai et al. [33] using TA98 with S9 activation.

^fFrom EPA web site for animal carcinogenicity study.