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Structure-dependent lipid peroxidation by photoirradiation of pyrene and its mono-substituted derivatives

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Abstract

Pyrene, one of the most studied polycyclic aromatic hydrocarbons can damage biological macromolecules and cause toxicity when irradiated by light. The effect of substituents, 1-amino, 1-hydroxy, 1-nitro, and 1-bromo, on light-induced lipid peroxidation is studied. Degradation kinetics and photoproduct analyses were conducted to test how these substituents affect the photoreaction. All five compounds have different photodegradation rates, and these rates parallel their light absorptivity. Four out of the five compounds induce lipid peroxidation when irradiated with UVA light; whereas, 1-aminopyrene causes minimum or no lipid peroxidation. The relative amount of lipid peroxidation caused is: 1-Bromopyrene > Pyrene > 1-Nitropyrene $\approx$ 1-Hydroxypyrene > 1-Aminopyrene. This relative lipid peroxidation is dependent on structure due to the following factors: light absorptivity, relative rates of the competing processes in the excited states, nature of the photoreaction, and nature of the photoproducts.

Keywords

Mono-substituted pyrene; photoreaction rate; UVA radiation; lipid peroxidation

Introduction

It is estimated that more than 5 million pounds of polycyclic aromatic hydrocarbons (PAHs) were released into the environment ^[1]. PAHs are a class of persistent environmental mutagenic contaminants that are mainly produced from the incomplete combustion of matters such as fossil fuels, foods, tobacco smoke, and other sources ^[2–5]. They are thought to induce cancerous tumors, primarily in the lungs, bladder, and skin ^[3, 6, 7]. PAHs' deleterious threats are not merely limited to metabolic activation, but photochemical as well ^[8–12]. Particularly, concomitant exposure to PAHs and light can lead to the generation of reactive intermediates ^[8] such as free radicals and reactive oxygen species ^[13], which may react with DNA, proteins, and lipids to cause various types of damages including lipid peroxidation ^[14–20].

Lipid peroxidation can become detrimental in human because they are associated with conditions including early liver problems, inflammation, atherosclerosis, and cancer ^[21–23]. Pyrene, one of the most widely studied and one of the most abundant PAHs, was found to generate light-induced lipid peroxidation in a previous study ^[18]. Studies on various structurally related PAHs, such as ethylchrysenes and methylbenz[a]anthracenes ^[16–19, 24], showed that light induced lipid peroxidation depends on PAH structure.

In this study, we report the light-induced lipid peroxidation by pyrene and its 1-amino, bromo, hydroxy, and nitro substituted derivatives (Figure 1). The level of light-induced peroxidation and photochemical reactions are compared, and a structure-activity relationship is discussed.

1-Hydroxypyrene (1-HP) is the main metabolite of pyrene [25–29]. 1-HP is detected in urine, and is hence often used as a biomarker for PAH exposure [5, 25, 27, 30]. Additionally, 1-HP can be both acutely toxic and genotoxic [31, 32]. Nitro-PAHs, including 1-NP, can be directly formed by the incomplete combustion of petrochemicals; other nitro-PAHs, though a minor process, can also be indirectly generated in the atmosphere from the reaction of a PAH with NO_2 or NO_3^- [33–35]. Nitro-PAHs have been found to be more mutagenic than their parent PAHs [33, 34, 36–40]. 1-NP can be reduced by nitro-reductases to 1-aminopyrene (1-AP) in living systems [41, 42]. Therefore, 1-AP is also included in this study. Most aromatic amines can be found within industrial chemicals, crude oil, and coal products and they can easily become widespread in the environment [43–45]. 1-AP can be oxidized into 1-hydroxyaminopyrene, a mutagenic intermediate, by cytochrome P450 enzymes [46, 47]. Unlike 1-NP, 1-AP was not found to induce cancerous tumors [36]. Although 1-bromopyrene (1-BP) occurs less abundantly compared to other compounds, it is chosen for comparison purposes.

Materials and Methods

Pyrene, 1-AP, 1-BP, 1-HP, 1-NP, methyl linoleate (ML), sodium azide (NaN_3), and potassium iodide (KI) were purchased from Sigma-Aldrich (St. Louis, MO). The PAHs were with a purity of at least 96% and used without further purification. The methanol used was spectroscopic grade, and the water used (18 Ω) was deionized by a Barnstead Nanopure Infinity water deionization system (Dubuque, IA). UV spectra were attained from a CHEM USB4-UV-Vis (Ocean Optics, Winter Park, FL).

Photochemical Reaction

Pyrenes (50 μM) in methanol were prepared from the methanol stock solution stored in the refrigerator. Two milliliters of the 50 μM pyrenes in pure methanol was irradiated with UVA light for a total of 30 min in a quartz cuvette (10×35 mm). The light source was a model TL-365R 15 W Spectroline UVA transilluminator (Westbury, NY) that produces an emission band at 365 ± 25 nm. The UVA irradiance is 5.8 mW/cm^2 , measured with a Model PMA 2100 Radiometer (Solar Light, PA). An absorbance spectrum was obtained at 5 min intervals using UV-Vis spectrophotometer. The wavelength used for each compound to determine the first-order reaction kinetics was: pyrene: 330 nm; 1-AP: 360 nm; 1-BP: 346 nm; 1-HP: 350 nm; 1-NP: 400 nm. The photochemical reaction kinetics was analyzed based on a first-order reaction, $\ln(A_0/A_t) = kt$, where A_0 and A_t refer to the absorption intensity of the pyrenes at irradiation times of 0 and t min, respectively; k is the first-order rate constant. The photodegradation rate was determined by plotting $\ln(A_0/A)$ vs irradiation time, where the slope was the rate constant, k . The degradation half-life of the compounds was calculated by $t_{1/2} = 0.693/k$.

Photo-induced lipid peroxidation

Lipid peroxidation experiments were carried out using 0.2 mM solution of the pyrenes in the presence of 100 mM ML in methanol. Each reaction was irradiated with the UVA light for a total of 60 min in a quartz cuvette. Samples were collected at 20 min intervals and detected using high-performance liquid chromatography (HPLC), a Shimadzu SCL-10A VP HPLC system (Shimadzu Corporation, Houston, TX). The peak areas were converted to ML

hydroperoxide concentrations based on the molar extinction coefficient at 235 nm as reported by Gibian and Vandenberg [48]. HPLC analyses were accomplished by injecting 10 μ L of the reaction mixture after each irradiation time. The column was a reverse phase ODS column, 250 $\times$ 4.6 mm, 5 micron (Phenomenex, Torrance, CA). The mobile phase was isocratic 10% water in methanol (v/v). For 1-NP, it was 15% water in methanol for a better separation. The flow rate was set at 1.0 mL/min. The signals were monitored at 235 nm. The experiment was conducted in triplicate. All results were analyzed using an Analysis of Variance (ANOVA) and the Tukey t-test to distinguish the differences between the Pyrene derivatives available through the Statistical Analysis Software SAS version 9.1. These tests α -level = 0.05.

Lipid Peroxidation in the Presence of Quenchers

NaN₃ or KI (50 mM) were incorporated into the solution mixture of 0.2 mM pyrenes and 100 mM ML in methanol. Each was irradiated with UVA light for 30 min in a quartz cuvette. Samples were collected and detected by HPLC analysis. The experiment was conducted in triplicate. All results were analyzed using an Analysis of Variance (ANOVA) and the Tukey t-test to distinguish the differences between the pyrene derivatives available through the Statistical Analysis Software SAS version 9.1. These tests α -value = 0.05.

Results and Discussion

Absorption Spectra

Pyrene and the four chosen 1-substituted Pyrenes all absorb light in the UV region with some extending into the visible region (Figure 2). The addition of a substituent, relative to Pyrene, results in a red-shifted spectrum, with the least and greatest shifts being due to the bromo and nitro groups, respectively. Pyrene has seven bands extending from 200–360 nm (238, 250, 260, 271, 304, 317, and 332 nm), while 1-BP has the same bands, but each red-shifted to 242, 255, 265, 273, 314, 329, and 343 nm, respectively. These seven bands can be further grouped into three main bands: 235–242, 250–271, and 304–343. 1-HP has six bands (241, 267, 277, 347, 365, and 385 nm). While the first two main bands at 241 and 267–277 are similar to that of 1-BP, the longer wavelength bands (347, 365, and 385) are not only red-shifted, but also have completely different shapes, indicating the effect of the extended conjugation of the hydroxyl group. The absorbance spectra of 1-NP and 1-AP have the same short wavelength bands at 235 nm, but the two other main bands are further red-shifted. In addition, the two longest wavelength bands lose the finger-print features. The relative absorbance of the UVA light sources (I_{rel}) of these compounds were determined and listed in Table 1.

Photochemical Reaction

With the exception of 1-AP, photodegradation kinetics of these Pyrenes were determined and calculated based on their absorbance obtained within the first 30 min of irradiation. Upon light irradiation, the 1-AP absorption decrease appeared to be small compared to that of 1-HP and 1-NP. HPLC analysis showed that new products were formed, and the absorbance spectra of 1-AP and its photoproducts were similar (Figure 4). Therefore, the degradation rate for 1-AP was determined by HPLC analysis. The 1-AP peak completely disappeared after 30 min of irradiation based on HPLC chromatogram. From this analysis, the degradation rate for 1-AP is $9.55 \times 10^{-2} \text{ min}^{-1}$ and the half-life is 7.3 min.

As shown in Figure 3, the photodegradation rates are different for all five compounds. 1-AP has the fastest photodegradation while Pyrene and 1-BP degrade very slowly. Both 1-HP and 1-NP degrade moderately. All of these UV-Vis analyses were confirmed by HPLC

analysis (Not shown). Table 1 lists the first-order rate constants and half-lives obtained from the photodegradation in methanol.

The absorption spectra and the degradation rates of all five compounds are substantially different. The light source used has an emission band at 365 ± 25 nm. These compounds' relative absorbance (I_{rel}) was determined by calculating the area under the absorption spectra within this region (340 – 390 nm). Thus, the calculated relative absorbance is: 1-AP > 1-HP > 1-NP > 1-BP > Pyrene (Pyrene's relative absorbance was defined as 1.0 for comparison purposes). These relative absorbance values parallel the degradation rates. The absorbance spectrum of 1-BP is slightly more red-shifted than Pyrene, thus contributing to the higher relative absorbance value. Pyrene and 1-BP both have the lowest relative absorbance because they do not display an extensive red shift, and comparatively speaking, they have the smallest photodegradation rates. This clearly indicates that light absorption is crucial for their degradation.

Another reason for the difference in degradation rates is the nature of the substituents. For example, the amino and hydroxyl groups in the excited state Pyrene can be readily oxidized, while the nitro group can either rearrange or be replaced by a substituent [8, 49–53]. 1-AP, 1-HP, and to a lesser extent, 1-NP's ability to absorb UVA light enables them to change the electronic distribution about the nucleus, employing bond breaking and creation [54]. This was demonstrated previously that 1-AP can be oxidized into 1-hydroxyaminopyrene, 1-nitrosopyrene, 1-nitropyrene, and 1-amino-x-hydroxypyrene [52]. The photoproducts of 1-HP are 1,6- and 1,8-pyrene quinones and a pyrene dimer [53], and the photo-induced reactions of 1-NP include pyrene, 1-HP, hydroxynitropyrenes, and pyrenediones [49, 50, 55]. Compared to the other 1-substituted Pyrenes, 1-NP was the least soluble in methanol [51]. In addition to the structural dependence, it has also been shown that the photoreaction of nitro-PAHs is dependent upon solvent polarity [49, 51]. For example, 1-NP photo-degrades faster in less polar and better soluble solvents such as chloroform.

Lipid Peroxidation

Upon UVA irradiation, ML can be transformed into *cis* and *trans* isomeric ML 9- and 13-hydroperoxides [13, 56]. Light-induced lipid peroxidation by Pyrene, 1-AP, 1-BP, 1-HP and 1-NP was monitored by HPLC (Figure 4). Each of the compounds was dissolved in methanol and irradiated by the UVA light, and a sample was taken at 0, 20, 40, and 60 min irradiation time for HPLC analysis. With the exception of 1-NP, the mobile phase used was 90% methanol and 10% water. At 90% methanol, 1-NP and ML hydroperoxides are not well-separated. Changing the mobile phase to 85% of methanol achieved good separation.

The amounts of ML hydroperoxides were calculated based on their HPLC peak area monitored at 235 nm [16, 17, 24]. Figure 5 plots the amount of ML hydroperoxides versus irradiation time during the 60 min of irradiation. 1-AP and 1-HP are nearly completely degraded within 60 min of irradiation based on their degradation half-lives of 7.3 min and 27.1 min, respectively (Table 1). It is also worth noting that neither Pyrene nor 1-BP degrades substantially during the 60 min of irradiation.

In comparison to the control, all compounds, except 1-AP, generate ML hydroperoxides (Figure 5). 1-BP generates the greatest amount of ML hydroperoxides. Pyrene is similar to 1-BP, but with less amounts of ML hydroperoxides, while the other compounds are less than Pyrene. It appears that the ability to generate lipid peroxidation is dependent on both the structure and these compounds' photoreaction rate.

The rate at which 1-HP produces ML hydroperoxides increased steadily, similar to both Pyrene and 1-BP until 40 min, where it levels off. The half-life for 1-HP is 27 min, and this leveling off is due to the near completion of degradation of 1-HP. It is known that the photoproducts of 1-HP are 1,6- and 1,8-pyrene quinones and a Pyrene quinone dimer [53], and these quinones can also induced lipid peroxidation [16]. The leveling off at 40 min for the 1-HP induced lipid peroxidation is because the quinones, though formed as photoproducts, were not produced in significant amounts as it is indicated in the HPLC chromatograms in Figure 1S (supplementary materials). Similarly, 1-AP does not generate significant amount of ML hydroperoxides due to its relatively fast photochemical reaction, producing sequentially 1-hydroxyamino, 1-nitroso, and 1-NP [52]. It means that these photoproducts of 1-AP are not causing significant amount of lipid peroxidation.

In contrast, the initial rate at which 1-NP generates lipid peroxidation was slower compared to Pyrene, 1-BP, and 1-HP, but there is a significant increase in lipid peroxidation at 40–60 min. The half-life of 1-NP degradation is 43 min. The sharp rise in lipid peroxidation suggests that the photoproducts of 1-NP are formed after 40 min of irradiation and they are more photoactive than 1-NP itself. The nitro group in 1-NP is not co-planar to the Pyrene ring [57]. Nitro-PAHs with none co-planar nitro groups are known to undergo photochemical oxidation of the aromatic ring [51, 58]. Photoreaction products of 1-NP include Pyrene, 1-HP, hydroxynitropyrenes, and pyrenediones [50, 55]. These photoproducts, at least as it is known for 1-HP in this study, are more photoactive in causing lipid peroxidation.

Involvement of Oxygen/ $^1\text{O}_2$

Quenchers, NaN_3 and KI, were used to determine whether reactive oxygen species (ROS) are involved in the induction of lipid peroxidation. NaN_3 is a singlet oxygen scavenger, while KI is a singlet state quencher. Samples were irradiated for a set time of 30 min. After taking into consideration the effects from the control, it is found that Pyrene, 1-BP, and 1-HP are quenched the most by NaN_3 (Figure 6). 1-BP and 1-NP are the most responsive to KI quenching. Therefore, it seems that all of the studied Pyrenes can generate singlet oxygen. We believe that the heavy atom effect of the bromo group in 1-BP is the main reason for the more pronounced quenching effect. Specifically, the bromo atom promotes intersystem crossing and produces a higher triplet state population with a quantum efficiency of at least two orders of magnitudes higher than Pyrene [59], thus creating more lipid hydroperoxides.

ROS species, such as singlet oxygen, are highly reactive. Singlet oxygen can create damages by directly reacting with biological macromolecules or through the formation of longer-lived hydroxide radicals [21, 22, 60, 61]. It is postulated that disruption and damage to macromolecules by ROS can lead to deleterious effects, such as tumor formation [23, 62]. Adverse effects of lipid peroxidation can be just as detrimental in humans as it is within animal systems. For instance, lipid peroxidation caused by ROS can be associated with conditions such as early aging, liver problems, inflammation, atherosclerosis, ischemia, and cancer [22, 23].

Structure-Activity Relationship

Pyrene and 1-AP, 1-BP, 1-HP, and 1-NP can all absorb UVA light leading to lipid peroxidation. As determined previously on PAHs including Pyrene, both singlet oxygen and superoxide are involved in lipid peroxidation caused by light irradiation of PAHs [13, 16–19, 24]. Generally, singlet oxygen is the main reactive species leading to lipid peroxidation for most PAHs we tested. It is also the case with the mono-substituted Pyrenes in this study. In addition, 1-HP was also shown to induce DNA single strand cleavage via singlet oxygen [32]. The amounts of peroxidation of these compounds depend on the

substituent, which governs the photochemical processes upon light excitation as shown in Scheme 1.

Upon absorption of light, Pyrene or its mono-substituted derivatives (represented by P) is promoted to the excited singlet state, 1P . The 1P can undergo several competing processes including fluorescence (K_f), nonradiative decay (K_{nr}), photochemical reaction to form photoproducts, and intersystem crossing (ISC) to reach the excited triplet state, 3P . Processes such as photoreaction (K_1 and K_2) and phosphorescence (K_p) can occur from 3P , but it can also react with ground state oxygen to produce singlet oxygen (1O_2), which can react with ML to produce ML hydroperoxides. Some photoproducts can also go through the similar light activation processes, leading to lipid peroxidation.

Thus, there are several factors governing the light-induced lipid peroxidation by Pyrenes that are responsible for the structural dependence: light absorptivity, relative rates of the competing processes, and the photochemical reactions. 1.) Light absorptivity: As seen in Table 1, the photodegradation rate is parallel to light absorptivity. More efficient light absorption can produce more excited state molecules leading to lipid peroxidation. 2.) Rates of competing processes: Competing processes leading to the triplet state include ISC versus K_{nr} , K_f , and singlet state photoreaction. This is seen in 1-BP since the presence of the bromine atom promotes intersystem crossing, resulting in two orders of magnitude higher triplet quantum yield in 1-BP versus Pyrene [59]. This is also seen in 1-AP, whose photodegradation half-life is only 7.3 min. Therefore, 1-AP is not causing much lipid peroxidation during the 30 or 40 min of irradiation since most of it was degraded. 3.) Photochemical reactions: Photoreactions lead to degradation (destruction of the parent compound) or generate products that can cause no, less, or more lipid peroxidation. For 1-HP, upon light irradiation, it degrades quickly and forms small amounts of quinones [32, 53]. This explains why the lipid peroxidation levels off after 40 min of irradiation. For 1-AP, upon UVA irradiation, it leads to the formation of 1-hydroxyamino, 1-nitroso, and 1-nitropyrene and some smaller fragmented molecules quickly [52], causing no lipid peroxidation. Conversely, 1-NP itself is a weak inducer of lipid peroxidation, but its photoproducts are stronger lipid peroxidation inducers, resulting in the ramping up of lipid peroxidation observed from 40–60 min of irradiation. Coincidentally, one of the photoproducts of 1-NP is 1-HP [49], which is a good lipid peroxidation inducer. As a result of the combination of factors, the relative light-induced lipid peroxidation is: 1-BP > Pyrene > 1-NP $\approx$ 1-HP > 1-AP.

Finally, it is also worth noting that solvent polarity plays an important role on the photochemical reaction rate of PAHs, such as it was shown with nitro-PAHs [51]. Since the lipid peroxidation experiments were carried out in methanol, it can be inferred that the amount of lipid peroxidation induced by these Pyrene derivatives may be different in other solvents, and the relative ability of these compounds causing lipid peroxidation may be altered.

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Figure 1.
Structures of pyrene and its mono-substituted derivatives.

Figure 2.
The absorbance spectra of 50 μ M pyrene and its derivatives in methanol.

Figure 3.

Photodegradation rates of 50 μM 1-aminopyrene, 1-hydroxypyrene, 1-nitropyrene, pyrene, and 1-bromopyrene. The degradation rate for 1-AP was calculated from the peak area using HPLC analysis, while the others were based on their absorbance changes.

Figure 4.
Representative chromatograph of ML peroxide formation and absorption spectra for 1-AP and 1-BP.

Figure 5.
Time-dependent ML Peroxide Formation by Pyrenes.

Figure 6.
Effect of NaN_3 and KI on light-induced peroxidation of ML by pyrenes after 30 min of irradiation (n=3).

Scheme 1.

Light excitation of pyrene and its mono-substituted derivatives (represented by P) leading to lipid peroxidation.

Table 1

Pyrene (50 μ M in methanol) derivatives' degradation rate constants and half-life values.

PAH	K (min^{-1})	Hal-life (min)	I _{abs} (rel)
Pyrene (330 nm ⁺)	1.60×10^{-3}	433	1.0
1-Bromopyrene (346 nm ⁺)	1.40×10^{-3}	495	6.7
1-Nitropyrene (400 nm ⁺)	1.60×10^{-2}	43.3	8.9
1-Hydroxypyrene (350 nm ⁺)	2.56×10^{-2}	27.1	13.9
1-Aminopyrene (360 nm ⁺)	$9.55 \times 10^{-2*}$	7.3 [*]	17.3

⁺The wavelength used for each compound to determine the first-order reaction kinetics.

^{*}Determined by HPLC method.

Table 2

UVA light-induced ML hydroperoxides (mM) in the presence of Pyrenes (0.2 mM), expressed as means $\pm$ SD (n = 3). The ML hydroperoxide concentrations were obtained after zeroing the ML background.

Samples	0 min	20 min	40 min	60 min
Control	0.0 $\pm$ 0.0	1.1 $\pm$ 0.7	2.1 $\pm$ 0.9	3.0 $\pm$ 1.8
Pyrene	1.9 $\pm$ 0.8	7.1 $\pm$ 0.4	12.1 $\pm$ 1.0	18.1 $\pm$ 1.4
1-AP	1.2 $\pm$ 0.0	2.0 $\pm$ 0.4	2.2 $\pm$ 0.5	3.3 $\pm$ 0.3
1-BrP	1.2 $\pm$ 0.7	9.3 $\pm$ 0.6	18.6 $\pm$ 1.9	27.6 $\pm$ 2.8
1-HP	0.8 $\pm$ 0.2	5.8 $\pm$ 0.6	10.4 $\pm$ 1.3	12.4 $\pm$ 1.0
1-NP	1.3 $\pm$ 0.7	5.4 $\pm$ 1.0	7.9 $\pm$ 1.9	13.8 $\pm$ 2.3