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Detection of Polycyclic Aromatic Hydrocarbon-DNA Adducts in Placental DNA Samples by Room-Temperature Solid-Matrix Phosphorescence

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Abstract: This is the first attempt for the direct detection of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in human placental DNA samples by solid-matrix phosphorescence (SMP). Six samples were investigated, and SMP emission spectra and the corresponding second derivative SMP spectra were obtained for all the samples. Numerous excitation and emission wavelengths were studied for detecting PAH-DNA adducts. Second derivative SMP spectra indicated the presence of PAH-DNA adducts, whereas the longer SMP emission region proved fruitful for detecting adducts in the placental DNA samples. The SMP results for the samples strongly implied that a variety of PAH-DNA adducts could be present.

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Keywords: Human placental DNA, polycyclic aromatic hydrocarbon-DNA adducts, solid-matrix phosphorescence

INTRODUCTION

The ability to characterize various types of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in human samples provides an important exposure metric in studies of cancer epidemiology and cancer risk. Polycyclic aromatic hydrocarbons result from various reactions, such as the combustion of fossil fuels or charring of organic matter. They appear in charbroiled foods, coal tar, soot, tobacco smoke, and other materials (Baum 1978; Yang and Silverman 1988; Dabestani and Ivanov 1990). Once ingested, inhaled, or absorbed, PAH may be metabolized to highly reactive electrophiles that form carcinogen-DNA adducts. Therefore, they provide a measure of the biologically effective dose of a carcinogen. In this work, PAH-DNA adducts in placental DNA samples were of interest as a surrogate biologically effective dose metric.

Analysis of placental DNA adducts has an important role in biomonitoring of human PAH exposures because the window exposure is short and well defined, <40 weeks. The first direct physicochemical evidence that environmental exposure to benzo[a]pyrene (B[a]P) samples can result in formation of benzo{a}pyrene-trans-7,8-dihydrodiol-9,10-epoxide (BPDE)-DNA adducts in vivo in human placenta was reported by Manchester et al. (1988). Human placental samples were treated with endonuclease and subjected to immunoaffinity chromatography using anti-benzo[a]pyrene-diol epoxide-DNA antibodies. Materials captured by the antibodies were hydrolyzed to release PAH-tetrohydrotetrols, which were fractionated by high performance liquid chromatography (HPLC), and BP-tetrol was identified by mass spectrometry and synchronous fluorescence spectroscopy (SFS) (Manchester et al. 1990). Weston et al. (1989) defined the PAH-specificity of antibodies for multiple PAH-DNA adducts and reported the utility of second derivative SFS to detect tetrols derived from the same placental samples. Immunoaffinity chromatography, SFS, and ^{32}P-postlabeling analysis were combined to investigate human placental DNA samples that were known to contain BPDE-DNA adducts (Manchester et al. 1990).

Others have studied PAH-DNA adducts using chemically less specific assays. The details of a fiber optic antibody-based fluoroimmunosensor for DNA adducts in human placenta samples was published by Vo-Dinh et al. (1991). Using this method, tetrols were also detected. The detection and quantitation of PAH-DNA adducts in maternal blood and newborn blood was reported by Perera et al. (2004). Tetrols were

determined by HPLC and fixed wavelength fluorescence spectrometry. Sanyal et al. (2007) discussed DNA adducts in human placenta exposed to ambient environment and passive cigarette smoke during pregnancy. The PAH adducts were assayed by ELISA using polyclonal antibody against benzo[a]pyrene-diol-epoxide-DNA in placental DNA. The metabolism of benzo[a]pyrene in vitro in human placental tissues exposed to active maternal cigarette smoke was reported by Sanyal and Li (2007). The metabolites formed were determined by extraction and HPLC.

In this work, room-temperature solid-matrix phosphorescence (SMP) was employed for the first time for the intact detection of PAH-DNA adducts in six placental DNA samples. This is a sensitive, selective, and a simple analytical approach for the detection and quantitation of a variety of aromatic species (Vo-Dinh 1984; Hurtubise 1990).

EXPERIMENTAL

Reagents and Placental Samples

The reagents employed have been discussed previously (Thompson and Hurtubise 2006, 2007). The human placental samples were obtained from E. D. Bowman (National Cancer Institute, Bethesda, MD) and D. K. Manchester (Children's Hospital in Denver, CO). The DNA was prepared as previously described (Manchester et al. 1988). The history of maternal tobacco smoking during the pregnancies was known (Table 1).

Apparatus and Software

The SMP data were obtained using a Perkin Elmer LS-50B spectrometer (Norwalk, CT). Spectra were taken with the excitation slits set at 3 nm,

Table 1. Adducts in placental samples based on excitation wavelengths[a]

Sample	Maternal Tobacco Smoking	347 nm	378 nm	390 nm
PD022	yes	−	+	+
PD025	yes	+	−	ND[b]
PD032	yes	+	+	+
PD033	no	+	−	ND[b]
PD036	yes	−	+	+
PD066[c]	no	+	+	+

[a] + indicates that adducts were detected, − indicates that adducts were not detected.

[b] ND = no data.

[c] For PD066 excitation wavelengths of 345 nm and 380 nm were used.

the emission slits set at 20 nm, $T_d = 0.1$ ms, $T_g = 9.9$ ms, and PMT voltage set at 775 volts. This system is designed with a pulsed source and gated detector and had a spectral resolution of ± 1 nm. The pulsed source was a xenon flash lamp pulsed at line frequency. The pulse width at half peak height is less then 10 μs and has a power equivalent to 20 kW for 8 μs duration. The instrumental parameters were controlled by the Fluorescence Data Manager (FLDM) software. The data were analyzed using GraphPad Prism version 3.00 for Windows, GraphPad Software (San Diego, CA). The SMP derivatives were calculated using Grams/AI version 7.00, Thermogalactic (Waltham, MA).

Procedures

The first sample investigated was PD036, which was taken from a freezer and brought to room temperature under limited lighting. Then an aliquot of the solution (1 mL) was placed in a volumetric flask (2 mL). The original solution of PD036 had a concentration of 2.08 μg/μL, giving 2.08 mg of DNA in the flask. The aqueous solvent was evaporated in a stream of nitrogen. Then, MeOH:H_2 O (0.5 mL, 30:70) was added. The solution was allowed to stand until the DNA was completely dissolved. Samples for SMP were prepared by spotting $TlNO_3$ (25 or 100 μg) onto Whatman 1PS filter paper and then spotting a portion (9 μL) of the placental DNA solution. Details for spotting the samples have been published previously (Thompson and Hurtubise 2007) for dual adducted DNA samples in which one DNA sample was modified with both the diol epoxides of dibenzo[a,l]pyrene and benzo[a]pyrene. Prior to spotting any samples, the Whatman 1PS paper was exposed to ultraviolet (UV) light for 3 hours after the paper was developed in ethanol to move impurities to one end of the paper. The previous two steps minimized SMP background signals from the 1PS paper. The amount of DNA spotted was 37.4 μg.

Sample PD036 was investigated using various excitation and emission wavelengths. A second aliquot was taken from the original container of PD036. Two mL of the original solution (2.08 μg/μL) was taken to dryness in a stream of N_2 over the solution. The sample was then redissolved in MeOH:H_2O (0.5 mL of 30:70). The new solution for PD036 had a concentration of 8.32 μg/μL, which provided an adsorbed amount of 75 μg when 9 μL was spotted. The instrument settings were the same as described previously. Spectra of other samples, PD022, PD025, PD033, PD066, and PD032, were obtained with 18 μL of solution spotted directly from the sample container and spotted on the Whatman 1PS paper that had 100 μg of $TlNO_3$ adsorbed on it. The same instrumental settings were used as described in the previous section.

RESULTS AND DISCUSSION

SMP Excitation and Emission Spectra of a Placental DNA Sample (PD036) and Calf-Thymus DNA

Room-temperature SMP excitation and emission spectra for placental DNA (PD036, 36 μg and 75 μg) and calf-thymus DNA (36 μg) were obtained after the samples were adsorbed on Whatman 1PS paper with $TlNO_3$ (100 μg) (Fig. 1). The SMP excitation spectra for each sample had a band between 335 nm and 342 nm. For PD036 at high concentration (75 μg), two bands were evident (Fig. 1). Unmodified calf-thymus DNA samples at a high amount (75 μg) did not show the two bands (data not shown). The PD036 sample also showed a well defined band around 372 nm in the excitation spectra, but the calf-thymus DNA sample did not. This band at 372 nm was also observed for placental DNA samples PD022, PD025, PD032, and PD066 but not PD033 (data not shown). A general discussion of the properties of excitation spectra and experimental conditions for excitation spectra can be found in Lakowicz (1999).

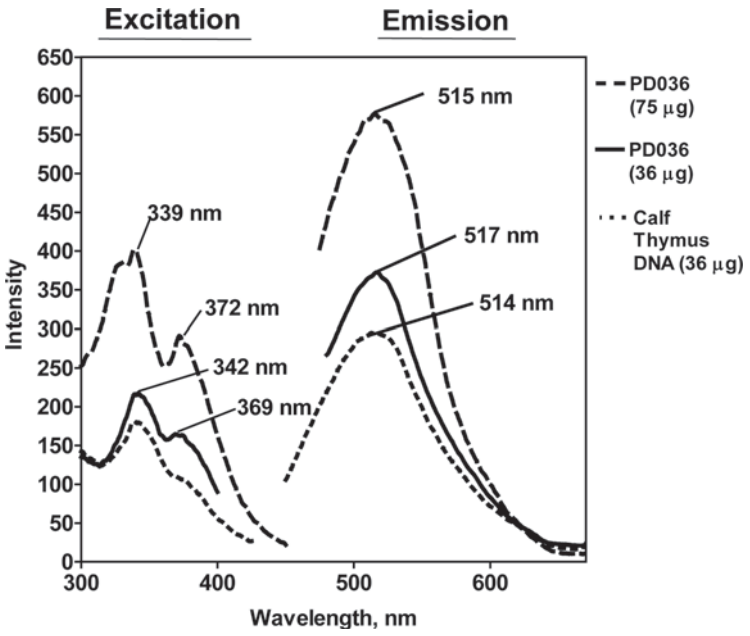

Figure 1. SMP excitation and emission spectra of placental DNA sample PD036 and calf-thymus DNA. All spectra were obtained with 100 μg of $TlNO_3$ on 1PS paper.

Figure 1 gives the SMP emission spectra for the two different sample sizes of PD036 (36 μg and 75 μg) and also for 36 μg of unmodified calf-thymus DNA with 100 μg of $TlNO_3$ adsorbed on the 1PS paper. The unmodified calf-thymus DNA sample shows a substantial SMP emission centered near 514 nm. However, for an unmodified calf-thymus DNA sample (18 μg) adsorbed on 1PS paper without $TlNO_3$, the SMP was very weak and the emission spectrum was broad and blue shifted about 13 nm (data not shown). The SMP intensity was 0.38 less than an 18 μg sample of unmodified DNA adsorbed on 1PS paper containing 25 μg of $TlNO_3$. These results and other results that we obtained show that unmodified calf-thymus DNA in the presence of the heavy-atom salt $TlNO_3$ gives enhanced SMP in the region of 514 nm, and the SMP emission spectrum is shifted to the red compared to an unmodified calf-thymus DNA sample without $TlNO_3$ present.

Figure 1 shows that any PAH-DNA adducts that emit SMP in the general region of 514 nm would be difficult to detect because of the SMP emission of unmodified DNA in this region. However, with an appropriate blank sample for placental DNA, the SMP contribution from the blank DNA could be potentially subtracted from the SMP emission spectrum of a modified DNA sample to obtain the SMP emission of the adducts. No appropriate blank placental DNA samples were available for this project. Thus, this aspect was not investigated. In general, the emission regions around 550 nm and beyond were emphasized in the investigation.

Previous Low-Temperature and Room-Temperature Phosphorescence of Nucleotides, Nucleic Acids, and Tetrols

There have been several reports of the low-temperature phosphorescence of nucleotides and nucleic acids (Bersohn and Isenberg 1964; Rahn, Shulman, and Longworth 1966; Rahn and Landry 1973; Boutilier, O'Donnell, and Rahn 1974). For example, Rahn, Schulman, and Longworth (1966) showed that the low-temperature solution phosphorescence of calf-thymus DNA gave a broad band with a peak at 448 nm, and the phosphorescence quantum yield was only 0.002. Also, Boutilier, O'Donnell, and Rahn (1974) reported the low-temperature properties of nucleosides (deoxyadenosine and deoxyguanosine) with Ag^+. With Ag^+ present, the phosphorescence intensity was enhanced and the emission spectra were shifted to the red.

In the preceding paragraph, all the studies were with solutions at low temperature. In this work, room-temperature SMP was employed because of its ease of use and the potential for very low detection limits

(Vo-Dinh 1984; Hurtubise 1990). It was shown by Vo-Dinh and Uziel (1987) that thallium acetate was more effective in enhancing the SMP of tetrol than lead acetate, silver nitrate, sodium bromide, and sodium iodide. They also reported the laser-induced room-temperature SMP detection of tetrol hydrolyzed from a sample of benzo[a]pyrene-DNA adducts using thallium acetate as a heavy-atom salt to increase the SMP. Also, Corley and Hurtubise (1994) showed that $TlNO_3$ was much more effective than $AgNO_3$ in enhancing the SMP of tetrols. In addition, Chu and Hurtubise (1996) demonstrated that $TlNO_3$ was very effective in increasing the SMP of (±)-trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-DNA adducts [(±)-anti-BPDE-DNA adducts] bonded to DNA. It was demonstrated for the first time by Li, Hurtubise, and Weston (1999) that a linear relationship could be obtained between the SMP and the percent modification of the (±)-anti-BPDE-DNA adducts adsorbed on 1PS paper containing $TlNO_3$. A limit of detection of 2 adducts in 10^7 bases was reported by them. Later, it was shown that one adduct in 10^7 bases could readily be detected using 1PS paper and 100 μg of $TlNO_3$ (Smith 2003).

SMP Excitation Spectra of the Placental DNA Samples

Because the placental DNA samples showed a significant excitation band around 372 nm, except for PD033, it is important to comment in more detail on this band. As stated, calf-thymus DNA does not show a well defined band at 372 nm. The heavy-atom effects for the room-temperature SMP of biologically important purines was discussed by Gaye and Aaron (1988). Also, Aaron, Campiglia, and Winefordner (1990) investigated the SMP of purine derivatives with either thallium acetate or thallium nitrate as a heavy-atom salt. They reported an SMP excitation wavelength of 273 nm for adenine and 278 nm for purine. There is no indication from our work with calf-thymus DNA, or the work of others, that an excitation band near 372 nm would appear in DNA. Thus, this band in the placental samples is from another source. We contend that it is most likely due to diol epoxides formed from PAH that bonded to DNA.

There are a number of PAH that have room-temperature excitation bands in the general ranges of 370 nm (Campiglia, Alarie, and Vo-Dinh 1996; Hagestuen, Arruda, and Campiglia 2000; Salinas-Castillo et al. 2004; Salinas-Castillo et al. 2005; Waris et al. 1989; Tucker et al. 1989; Tucker et al. 1991; Porro et al. 1973). For example, others (Waris et al. 1989; Tucker et al. 1989; Tucker et al. 1991) have used solution fluorescence and reported the excitation wavelengths for several PAH, including

large PAH (seven or more six-member rings) in the general range of 370 nm. In this work, an approximate range of excitation wavelengths from 365 to 385 nm was assumed to be acceptable as an excitation wavelength range from the literature that could be compared to the band near 372 nm in the placental DNA samples. Using the information in the literature, it was obvious that several PAH have excitation bands in the region of 365–385 nm that could be correlated with the excitation band around 372 nm in the placental samples, such as chrysene (four aromatic rings), benzo[k]fluoranthrene (four aromatic rings), benzo[a]anthracene (four aromatic rings), benzo[a]pyrene (five aromatic rings), indeno[1,2,3-cd]pyrene (five aromatic rings), benzo(g,h,i)perylene (six aromatic rings), anthanthrene (six aromatic rings), and 1,2,4,5-dibenzpyrene (six aromatic rings). It is postulated that the band around 372 nm in the placental samples is from one of these aromatic systems or from the ring system of another PAH that would have been around 372 nm.

It is important to note that the band near 372 nm became less intense and less defined spectrally after several cycles of thawing and refreezing of the samples. This was most likely due to some photodecomposition, even though every precaution was taken to minimize exposure to light. The loss of intensity and spectral properties is added evidence that the 372 band was related to PAH-DNA adducts because PAH-DNA adducts are known to be photosensitive (Geacintov et al. 1987).

Another heavy-atom salt, NaI, was also investigated to increase the SMP of the placental DNA samples, but it was found to be as less effective as $TlNO_3$. Also, with NaI, none of the placental samples showed the SMP excitation band at 372 nm. By investigating several salts for the solution fluorescence quenching of (±)-anti-BPDE-DNA adducts, Prusik et al. (1979) showed that I^- did not easily penetrate DNA, but Ag^+ could preferentially bind to DNA bases. The same should be true for Tl^+. For example, Howerton et al. (2001) reported a detailed study of the bonding of Tl^+ to bases in B-DNA. From our SMP results for the placental samples with Tl^+, it appears that the Tl^+ ions can penetrate the DNA and readily interact with the DNA bases and thus achieve close contact with PAH-DNA adducts.

Second Derivative of an Excitation Spectrum and a Detailed Study of an Excitation Spectrum

Second derivative spectroscopy is useful in reducing spectral and background interference (Weston et al. 1989; Thompson and Hurtubise 2007; Green and O'Haver 1974; O'Haver 1979). Figure 2 gives the second derivative excitation spectra for PD036 and calf-thymus DNA from 330

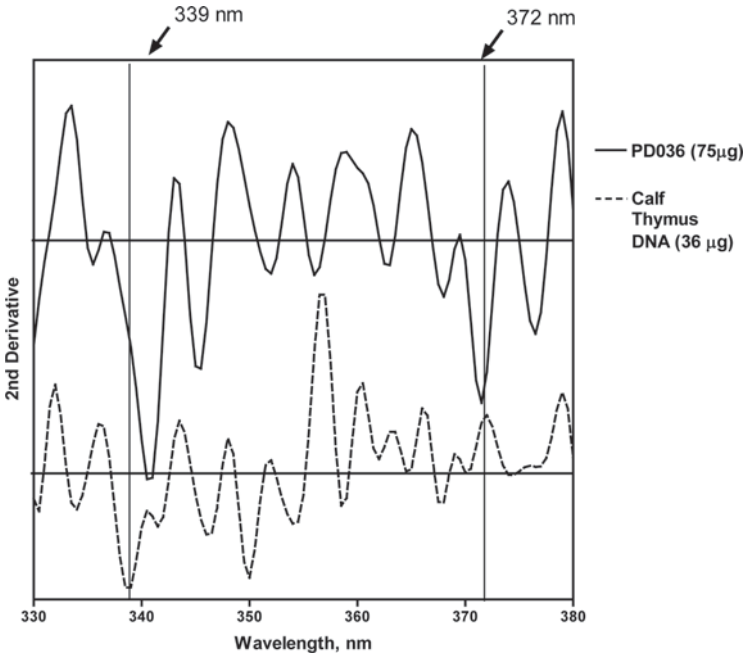

Figure 2. Second derivative SMP excitation spectra of PD036 [75 μg] (–) and calf-thymus DNA [36 μg] (– –) on Whatman 1PS paper with 100 μg of $TlNO_3$ obtained with $\lambda_{em} = 556$ nm.

to 380 nm. As Fig. 2 shows, the overall second derivative patterns for the two samples are very different. In the regions of 339 nm and 372 nm, the second derivatives of PD036 and calf-thymus DNA show significant differences. Figure 2 is further evidence that PD036 has been most likely modified with diol epoxides of PAH.

An investigation was carried out with sample PD066 in which SMP excitation spectra were recorded with emission wavelengths changed in 5 nm increments from 475 nm to 650 nm. The specific focus was on the excitation band near 372 nm. The SMP intensity of the excitation spectrum should be the largest when the emission monochromator is set at the maximum emission wavelength for the emitting PAH system (or systems). Figure 3 compares three of the excitation spectra obtained in the region from about 300 nm to 420 nm with the emission monochromator set at 630 nm, 610 nm, and 540 nm. It is clear from Fig. 3 that the intensity of the excitation band increases as the setting of the emission monochromator decreases. At a setting of 540 nm, the excitation band near 372 nm is relatively strong compared to the settings of 630 nm and

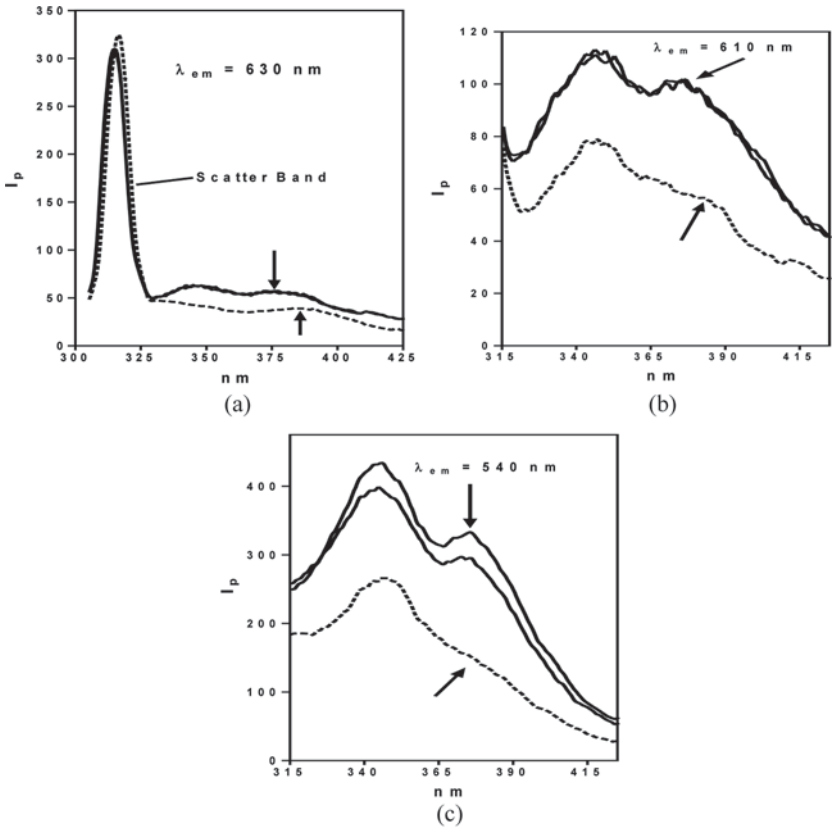

Figure 3. Excitation spectra obtained with 18 μL of PD066 (–) with 100 μg of $TlNO_3$ and blank calf-thymus DNA (– –), (a) $\lambda_{em} = 630$ nm, (b) $\lambda_{em} = 610$ nm, and (c) $\lambda_{em} = 540$ nm.

610 nm. This indicates that the emitting aromatic-ring system (or systems) has a strong emission around 540 nm.

One can speculate as to which aromatic ring system would have a strong emission around 540 nm with an excitation band near 372 nm. Because of instrument-to-instrument variation and different experimental conditions, a general guideline of ±15 nm was used for the 372 nm band and the 540 nm band for PAH aromatic systems from the literature. An examination from the phosphorescence literature for both room-temperature and low-temperature phosphorescence spectral properties for PAH gave the following PAH that have excitation and emission bands within the general guideline: fluoranthene, benz[b]fluoranthene, dibenz[a,c]anthracene, and dibenz[a,h]anthracene (Salinas-Castillo et al.

2004; Vo-Dinh and Hooyman 1979; Hagestuen and Campiglia 1998; Martin and Campiglia 2001).

SMP Emission Spectra and Second Derivative SMP Emission Spectra of Human Placental DNA Samples

Even though DNA shows a relatively strong SMP emission in the region of 514 nm (Fig. 1), several PAH have phosphorescence emission bands in the general range of 530 nm to 750 nm (Campiglia, Alarie, and Vo-Dinh 1996; Hagestuen, Arruda, and Campiglia 2000; Salinas et al. 2004; Salinas et al. 2005). Thus, this longer wavelength SMP spectral region was investigated in detail for SMP from diol epoxides of PAH bonded to the placental DNA samples. Using sample PD066 as a model system, SMP emission spectra were obtained using excitation wavelengths in the range of 250 nm to 395 nm at 5 nm increments. Even though DNA absorbs strongly in the general range 250 nm to 300 nm (Sinden 1994), this range of wavelengths was also investigated to determine if any SMP emission could be detected from the diol epoxides of PAH. To obtain the SMP emission spectra, it was decided to use primarily 347 nm, 356 nm, 378 nm, and 390 nm as excitation wavelengths to acquire the SMP emission spectra for the placental samples. Also, second derivative SMP emission spectra were obtained for most of the placental samples using the four excitation wavelengths. Because the SMP bands from the DNA adducts were superimposed in the SMP emission spectra of DNA (Fig. 1), it was somewhat difficult to exactly reproduce each replicate SMP spectra. The reproducibility for a given wavelength was from run-to-run about ± 8 nm. However, the overall spectral patterns were reproducible.

Representative SMP emission spectra and second derivative emission spectra of PD032 and PD033 will be discussed. Figure 4A shows the SMP emission spectra for PD032 and calf-thymus DNA excited at 378 nm. Figure 4B gives the corresponding second derivative SMP spectra of the samples. Figure 4A shows bands at 620 nm and 644 nm that do not appear in the calf-thymus SMP emission spectrum. In Fig. 4B, the second derivative spectra are presented. It is clear that the patterns for the second derivative spectra are different in several regions of the spectra for PD032 at this excitation wavelength compared to calf-thymus DNA. In particular, in the regions around 620 nm the two spectra are significantly different. Figure 5A shows the SMP emission spectra and second derivative spectra for PD033 using an excitation wavelength of 347 nm. It is evident from the emission spectra that two bands (625 nm and 640 nm) appear for PD033, but these bands are not in the calf-thymus DNA sample (Fig. 5A).

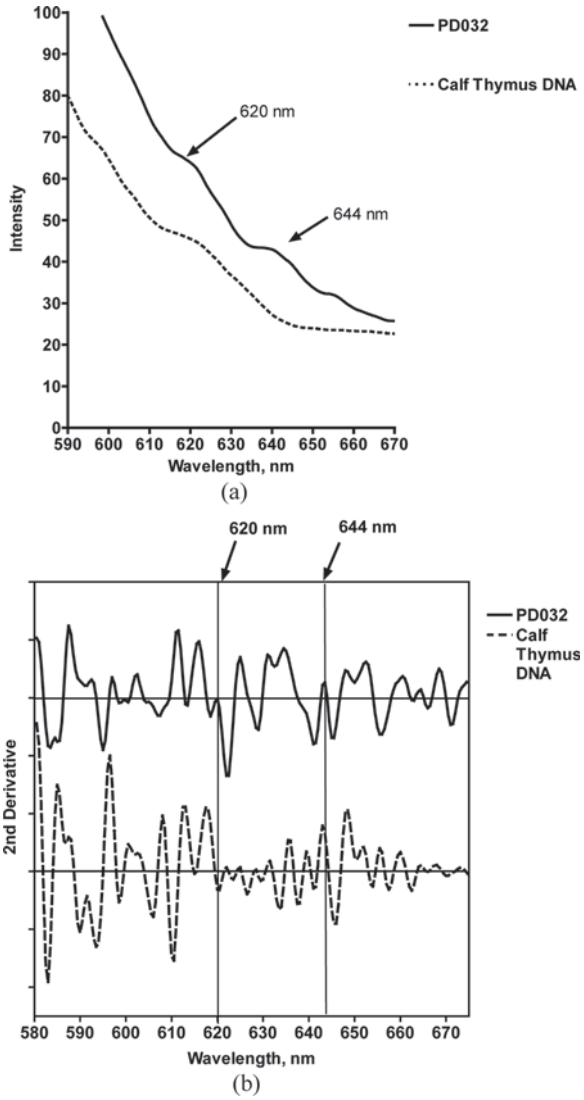

Figure 4. (a) SMP emission spectrum of PD032 [39.6 μg] (–) and calf-thymus DNA (– –) on Whatman 1PS paper with 100 μg of $TlNO_3$ obtained with $\lambda_{ex} = 378$ nm; (b) second derivative SMP emission spectra of PD032 [39.6 mg] (–) and calf-thymus DNA (– –) on Whatman 1PS with 100 μg of $TlNO_3$ obtained with $\lambda_{ex} = 378$ nm.

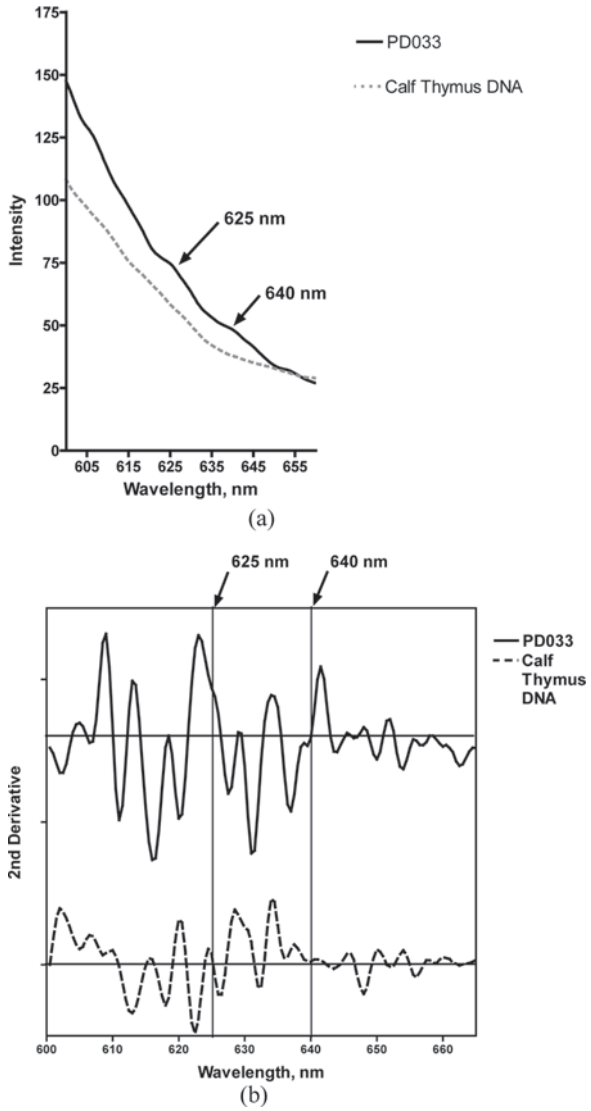

Figure 5. (a) SMP emission spectra of PD033 [22.5 μg] (–) and calf-thymus DNA (– –) on Whatman 1PS with 100 μg of $TlNO_3$ obtained with $\lambda_{ex} = 347$ nm; (b) second derivative SMP emission spectra of PD033.

A comparison of the second derivative spectra for PD033 and calf-thymus DNA in Fig. 5B show major differences, especially the regions of 625 nm and 640 nm. Again, this indicates the presence of DNA adducts.

Comparison of SMP Spectra and Second Derivative SMP Emission Spectra of the Six Placental Samples

A detailed study of the six placental samples was carried out by comparing the SMP emission spectra and the second derivative spectra obtained from primarily three excitation wavelengths (347 nm, 378 nm, and 390 nm). The six samples are discussed in order of increasing excitation wavelengths. Rather than discussing all of the emission spectra in detail only a brief summary of the results will be given.

For PD022, no SMP emission bands appeared with an excitation wavelength of 347 nm. It should be mentioned that an excitation wavelength of 345 nm was used for PD066, rather than 347 nm. Based on the emission results for PD025, PD032, PD033, and PD066, the presence of PAH-adducts were indicated. With sample PD036, an SMP band was present, and the second derivative spectra supported the presence of a band. However, the three SMP spectra were significantly shifted relative to one another, but the band shapes were the same. The bands had the following wavelength ranges: 625 to 645 nm, 640 to 657 nm, and 649 to 667 nm. The reasons for the shifts are not clear, but it could be related to how the DNA samples were distributed on the solid matrix. Sample PD066 was excited with 380 nm (rather than 378 nm), and a SMP band showed up from 640 to 670 nm. Based on the SMP emission results, PD022, PD032, PD036, and PD066 indicated the presence of adducts. The results obtained with an excitation wavelength of 390 nm showed that adducts are present in the PD022, PD032, PD036, and PD066 samples.

Table 1 summarizes the results obtained with the three excitation wavelengths. Depending on the excitation wavelengths used, the results in Table 1 suggest the presence of PAH-DNA adducts. Column two in Table 1 shows that there was no history of tobacco smoking for sample PD033 and PD066, but the SMP results indicated the presence of PAH-DNA adducts. It is possible that someone not exposed to maternal smoking could have PAH-DNA adducts in the placenta. For example, a mother could have been exposed to polluted air that contained PAH and inhaled the PAH.

Possible PAH Systems Emitting SMP

Based on the excitation wavelengths reported in this section (347 nm, 378 nm, and 390 nm), and phosphorescence emission spectral properties reported in the literature for PAH, it is possible to infer which PAH aromatic ring systems could emit in the general range (600 to 670 nm) of

SMP emission for the DNA placental samples. Because both excitation and emission wavelengths can vary as a result of experimental and instrumental conditions, it is assumed that the excitation and emission spectral wavelengths that we obtained could have an approximate range of ± 10 nm compared to the literature phosphorescence data. Also, it is assumed that the maximum excitation wavelength was not necessarily used in exciting the SMP from the DNA adducts in our work because there was no way of knowing beforehand what the maximum excitation wavelengths were.

Considering an excitation wavelength of 347 nm, the following PAH could emit in the 600–670 nm range: pyrene, dibenz[a,c]anthracene, dibenz[-a,h]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]-pyrene, anthracence, benzo[a]anthracene, fluoranthene, benzo-[a]pyrene, and benzo[g,h,i]perylene (Vo-Dinh 1984; Hagestuen, Arruda, and Campiglia 2000; Salinas-Castillo et al. 2005; Vo-Dinh and Hooyman 1979; Hagestuen and Campiglia 1998; Martin and Campiglia 2001). For an excitation wavelength of 378 nm, the subsequent PAH could emit in the 600–670 nm range: dibenz[a,c]anthracene, benzo[b]fluoranthene, benzo[k]-fluoranthene, indeno[1,2,3-cd]pyrene, anthracence, benzo[a]anthracene, fluoranthene, benzo[a]pyrene, and benzo[g,h,i]perylene (Vo-Dinh 1984; Hagestuen, Arruda, and Campiglia 2000; Salinas-Castillo et al. 2004; Salinas-Castillo et al. 2005; Vo-Dinh and Hooyman 1979; Hagestuen and Campiglia 1998; Martin and Campiglia 2001; Hagestuen and Campiglia 1999). With an excitation wavelength of 390 nm, the following PAH could emit in the range of 600–670 nm: benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, anthracence, benzo[a]pyrene, and benzo[g,h,i]perylene (Vo-Dinh 1984; Hagestuen, Arruda, and Campiglia 2000; Salinas-Castillo et al. 2004; Salinas-Castillo et al. 2005; Hagestuen and Campiglia 1998; Martin and Campiglia 2001; Hagestuen and Campiglia 1999; Campiglia, Bystol, and Yu 2006). It is clear that there are several possible aromatic ring systems in the placental DNA sample that would be revealed by SMP.

CONCLUSIONS

This investigation reports the first attempt at the intact detection of PAH-DNA adducts in placental DNA by SMP. The SMP emission detected is from aromatic systems not normally present in DNA. The SMP data strongly suggest that PAH-DNA adducts are emitting. It is not known which PAH systems are emitting, but the spectral regions of emission allowed speculations on which aromatic ring systems were emitting SMP. The research results open the door for further

PAH-DNA adduct research in human samples and confirms that it is important to consider the possibility that a variety of PAH ring systems could be bonded to DNA (Manchester et al. 1992).

Evidence for the presence of PAH-DNA adducts in placental DNA samples were the SMP emission in the spectral region where PAH emit SMP and the lack of definitive SMP from unmodified DNA. Also, the second derivative SMP emission spectra gave support for the PAH-DNA adducts. In addition, the changes in the SMP excitation spectra of the placental DNA samples, and the lack of similar changes in the excitation spectra of unmodified DNA as a function of emission wavelength, gave strong evidence for PAH-DNA adducts in placental DNA. Other possible candidates for SMP emission would be adducted heterocyclic aromatic amines, such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP) (Becher et al. 1988). However, a recent investigation in this research group of the solution low-temperature phosphorescence of eight heterocyclic aromatic amines showed that the SMP maximum emission wavelengths were in the region of 450 nm, which is in a much shorter wavelength region than the PAH-DNA adducts (Mendonsa 2001).

It should be mentioned that the SMP emission from the placental DNA samples also could be from nitro-PAH-DNA adducts. Some nitro-PAH are potent mutagens and can form nitro-DNA adducts (Umbuzeiro et al. 2008). Little work has been done on the phosphorescence of nitro-PAH, and no work has been done on the phosphorescence of nitro-DNA adducts. The solution low-temperature phosphorescence spectra of 22 nitro-PAH have been reported (Wolfbeis et al. 1983). Many of those nitro-PAH investigated have phosphorescence excitation and emission properties similar to the SMP properties observed from the placental DNA samples. For example, 1-nitropyrene has a phosphorescence excitation band at 374 nm and a phosphorescence emission band at 639 nm. Also, nitro-PAH have little or no fluorescence (Wolfbeis et al 1983). Thus, fluorescence spectrometry would not be useful in detecting nitro-PAH-DNA adducts. However, SMP should be very effective in detecting nitro-PAH-DNA adducts.

Little research has been done on the characterization of multiple-adducted human DNA samples. The SMP methodology developed in this research would be helpful in the characterization of multiple-adducted human DNA samples. The SMP research should be expanded to include the hydrolysis of the PAH-DNA adducts from DNA samples and the characterization of the aromatic hydrolysis products by SMP. This additional step would permit detailed conformation of which PAH systems are directly bonded to DNA. For example, Vo-Dinh and Uziel (1987) obtained a tetrol sample from the hydrolysis of a BPDE-DNA sample and characterized the tetrol by SMP. Also, Corley et al. (1995)

employed SMP for the identification and quantitation of tetrols derived from the acid hydrolysis of BPDE-DNA adducts from human lung samples.

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