Anal. Chem. **1999,** *71,* 4679−4683

Determination of Benzo[*a*]pyrene−DNA Adducts by Solid-Matrix Phosphorescence

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It has been demonstrated for the first time that linear relationships can be obtained between the solid-matrix phosphorescence (SMP) and the percent modification of benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE)−DNA adducts. The samples of DNA were modified with BPDE at levels of 5.0×10^{-3}, 1.0×10^{-3}, 5.0×10^{-4}, 1.0×10^{-4}, 5.0×10^{-5}, and 1.0×10^{-5}%. In addition, the changes in the SMP intensities of a given percentage of DNA adduct were investigated as a function of sample size, and linear relationships were also acquired. With the different percentages of modified DNA, very good reproducibility of the SMP signals was obtained. Data were acquired with both Whatman 1PS paper and 30% $TlNO_3$/sodium acetate as solid matrixes. The limit of detection for the BPDE−DNA adducts was 2 adducts in 10^7 bases for both Whatman 1PS paper and 30% $TlNO_3$/sodium acetate. In addition, it was shown that it would be important to develop a standard procedure for the preparation of the BPDE−DNA samples if different batches of DNA were used in the preparation procedure.

There is considerable interest today in the toxic effects of polycyclic aromatic hydrocarbons in the human body.[1] For example, benzo[*a*]pyrene (B[*a*]P) is a ubiquitous environmental contaminant and a potential human carcinogen. Essentially chemically inert, B[*a*]P is metabolically activated to a variety of oxygenated products, and its biological properties have been studied comprehensively in model biological systems.[2,3] Some of the products include the highly reactive, mutagenic, and tumorigenic diol epoxide derivatives.[4] It has been established that an important ultimate carcinogenic metabolite of B[*a*]P is benzo[*a*]-pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), which acts through the formation of covalent addition products in DNA.[5−7] Advances in human environmental cancer etiology increasingly rely on genotoxic or molecular dosimetry.[8] This report describes an innovative physicochemical approach to this significant problem.

Sensitive and simple methods are needed to directly detect and quantify the BPDE−DNA adducts because of the major importance of the adducts in cancer research. Some of the approaches developed to characterize and determine BPDE−DNA adducts are immunoassays,[8] synchronous fluorescence spectrometry,[9] radioactive ^{32}P post labeling,[10] GC/MS,[11] CE/MS,[12,13] a fluoroimmunosensor,[14] and fluorescence line-narrowing spectroscopy.[15,16] Several of these methods require special handling techniques such as liquid nitrogen or helium cooling and manipulation of radioactive materials. Also, many of the methods require expensive instruments, and most of the approaches described in the literature are indirect, in that the hydrolysis products from the BPDE−DNA adducts, 7,8,9,10-tetrahydrotetrahydroxybenzo[*a*]pyrenes (tetrols), are determined. Analytical methods that are simple, sensitive, and inexpensive for the direct determination of the BPDE−DNA adducts are greatly needed.

Solid-matrix room-temperature phosphorescence (SMRTP) has the advantages of simplicity, sensitivity, and low cost. Several researchers have demonstrated the usefulness of SMRTP for a variety of applications.[17−19] Recently, solid-matrix luminescence has been shown to be very beneficial for the characterization and

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10.1021/ac9905726 CCC: $18.00
Published on Web 09/11/1999

determination of tetrols.[20−26] For example, it was demonstrated that SMRTP with an α-cyclodextrin solid matrix was capable of identifying and quantitating tetrols derived from human lung fractions.[24] The limit of detection for tetrol was 0.4 fmol. In addition, a synchronous SMRTP method was developed for detecting tetrols at the subnanogram level in the presence of BPDE−DNA adducts.[26] In a detailed study, both solid-matrix room-temperature fluorescence (SMRTF) and SMRTP were employed to compare the luminescence properties of BPDE−DNA adducts.[27] In later work, it was shown that $TlNO_3$ was very effective in enhancing the SMRTP of BPDE−DNA adducts.[28] Several SMRTF and SMRTP methods were developed for the detection and characterization of BPDE−DNA adducts with Whatman No. 1 and Whatman 1PS filter paper.[29] For example, with Whatman 1PS filter paper, a unique method was developed for the SMRTF detection of adducts externally bound to DNA. Recently, new approaches were developed for the SMRTF and SMRTP determination of BPDE−DNA adducts at a relatively high level of modification (2.6%).[30] It was shown that SMRTP gave lower limits of detection than SMRTF, and the mass of DNA sample that was modified at 2.6% could be detected at a level of 1 ng by SMRTP. In this work, DNA was modified at very low levels, and the SMRTP properties of the BPDE−DNA adducts were used to demonstrate that the BPDE−DNA adducts could be determined at these low percentages of modification.

EXPERIMENTAL SECTION

Apparatus. A Perkin-Elmer LS-50B luminescence spectrometer was used to obtain all the phosphorescence data. The system is designed with a pulsed source and gated detector system. A delay time (t_d) of 0.5 ms and a gate time (t_g) of 9.5 ms was used for the phosphorescence measurements, and a typical cycle time was 32 ms. The excitation slit was set at 15 nm, and the emission slit of 20 nm was employed. The spectral band-pass for the luminescence spectrometer is determined by the slit setting. The excitation slits can be varied from 2.5 to 15 nm, and the emission slits can be varied from 2.5 to 20 nm in 0.1-nm increments. Thus, a spectral band-pass of 15 nm would be achieved with a slit setting of 15 nm. Wide spectral band-passes were used in this work to acquire strong SMRTP signals.

Two Delrin sample holders were custom-made to handle powders and filter paper samples. The lower half of a 12 × 12 × 50 mm^3 Delrin block was sectioned diagonally, and in the center of the section there was a depression. The two types of sample holders only differed in diameter and depth of the depression. For the powder samples, the depression was 6 mm in diameter and 1 mm in depth, and for filter paper samples, the depression was 3 mm in diameter and 0.5 mm in depth. The sample holders were designed so that the source radiation impinged at the center of the depression of a given holder when it was placed in the cell holder of the LS-50B. There was no need to align the sample because the Delrin sample holder fit tightly into the cell holder of the LS-50B luminescence spectrometer.

Preparation of BPDE−DNA Adducts. Six DNA samples were modified with BPDE as described earlier.[30] Two different batches of purified DNA were used in the modification experiments. Four DNA samples were modified with BPDE with the first batch of purified DNA. Then two more samples of DNA were modified with the second batch of DNA. Modifications of the six DNA adduct samples were conducted at extremely low levels, and direct determination of the percentage of modification via ultraviolet absorption spectrometry[31] was not feasible because of the lack of sensitivity of this technique for the samples. However, it is known that the percentage of modification of DNA samples is proportional to the relative amounts of BPDE and DNA used.[8,32] The percentages of modifications of the six DNA samples (5.0×10^{-3}, 1.0×10^{-3}, 5.0×10^{-4}, 1.0×10^{-4}, 5.0×10^{-5}, 1.0×10^{-5}) were calculated using ultraviolet absorbance data and DNA/BPDE ratios from other DNA samples that were modified at higher levels. The first batch of purified DNA in this work gave four samples with percentages of modifications of 5.0×10^{-3}, 1.0×10^{-3} , 5.0×10^{-4} , and 1.0×10^{-4}%. The second batch of purified DNA gave two samples with percentages of modifications of 5.0×10^{-5} and 1.0×10^{-5}. It should be mentioned that a DNA sample modified at 1.0×10^{-3}% is the same as 1 adduct in 10^5 nucleotides, which, in turn, is equivalent to 30 fmol of BPDE per microgram of DNA.

Experimental Procedures. Two solid matrixes were investigated: 30% $TlNO_3$/sodium acetate (NaOAc) powder and Whatman 1PS filter paper. The major experimental details for adsorbing the BPDE−DNA adducts on these solid materials were discussed previously.[29,30]

The six modified DNA stock solutions were all 2 μg/μL in 30% MeOH/H_2O. For the 30% $TlNO_3$/NaOAc solid matrix, the adsorption of the modified DNA was carried out as previously reported.[30] For Whatman 1PS filter paper matrix, 25 μg of $TlNO_3$ was first put on the paper disks. To obtain a relatively strong SMP signal from the adduct samples modified at such low levels, a sample size of about 36 μg of modified DNA was needed. Also, multiple spottings of the stock solutions onto the paper disks (under darkened lab conditions) were necessary to achieve the desired sample size. In addition, spectroscopic experimental details can be found in the literature.[30] All SMP measurements were made under dry nitrogen conditions.

RESULTS AND DISCUSSION

Modification of DNA Samples. Percentages of modification of the DNA samples at very low levels of BPDE were calculated on the basis of ultraviolet absorbance data and DNA/BPDE mass ratios for DNA samples that were modified at higher levels. For example, we previously reported SMP data for a DNA sample that was modified with BPDE at a level of 2.62%.[30] On the basis of the DNA/BPDE mass ratio for this sample, the mass ratios needed

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to modify DNA samples at 0.01, 0.05, 0.10, 0.50, and 1.00% were calculated. After the DNA samples were modified with BPDE using the calculated mass ratios, the percentage of modification of each sample was determined by ultraviolet spectrometry.[31] The observed values were 0.0098 ± 0.0001, 0.043 ± 0.003, 0.092 ± 0.002, 0.45 ± 0.01, and 1.09 ± 0.01%. Comparison of the previously calculated values with the expected percentage modifications gave the following respective percent errors: −2.0%, −14%, −8.0%, −10%, and 9.0%. Also, a plot of experimental percentage modification versus expected percentage modification for the five modified DNA samples gave a linear correlation coefficient of 0.996. In this work, the amounts of BPDE added to the six solutions for DNA modification were calculated on the basis of the results acquired for the five samples of DNA that were modified earlier (0.0098, 0.043, 0.092, 0.45, and 1.09%). In addition, the percentages of modified DNA for the samples in this work were calculated by assuming that the amount of BPDE that would react with DNA was linearly related to the mass of BPDE used as demonstrated by others.[8,32] Normally, DNA samples modified at very low levels (about 0.1−4.5 fmol BPDE per microgram of DNA) are quantitated using a tritium tracer. The modification levels are then determined from the DNA concentration and specific activity.[8] We do not have the capability of measuring [^{3}H] BPDE activity. Thus, we used the approach described above to determine the percent modification of the DNA samples. It is not possible to calculate the specific errors in the percentages of the modification of the DNA samples modified at very low levels. Therefore, if it is assumed that the errors are in the same range as for the samples modified at the expected values of 0.01, 0.05, 0.10, 0.50, and 1.00%. Then, it is possible to estimate the absolute errors in the percent modification of the DNA samples modified at very low levels by using the high (−14%) and low (9.0%) errors found for the DNA samples modified at the higher levels. For example, for a DNA sample with an expected modification of 0.005%, the estimated absolute errors in the percent modification are 0.0043 and 0.0055%. As another example, for a DNA sample with an expected modification of 1.0×10^{-5}%, the estimated absolute errors in the percent modification are 0.86×10^{-5} and 1.09×10^{-5}%.

Spectroscopic Data and Tetrols. Typical SMP excitation and emission spectra of a 0.005% modified BPDE−DNA adduct sample on 30% $TlNO_3$/NaOAc show that BPDE−DNA adducts give a SMP emission maximum at 612 nm (Figure 1). In this region, the SMP is far removed from scattered light, and background SMP signals are less than those at shorter wavelengths. The adduct samples modified at the other levels gave similar spectra both on 1PS paper and 30% $TlNO_3$/NaOAc. The SMP emission maximum wavelength of tetrol I-1 adsorbed on 30% $TlNO_3$/NaOAc under the same conditions as in Figure 1 was 4 nm shorter than that of the adduct. Also, the maximum of the phosphorescence excitation spectrum (351 nm) of tetrol I-1 adsorbed on 30% $TlNO_3$/NaOAc, under the same conditions as in Figure 1, was 4 nm shorter than that of the adduct. These are some of the results used to show that tetrols, which are the hydrolysis products of the adducts, were not present in the BPDE−DNA samples. The average SMP emission wavelength for samples of BPDE−DNA adducts (40 μg) modified at the first four levels occurred at 611 nm. For tetrol I-1 adsorbed on 30% $TlNO_3$/NaOAc, the average SMP emission wavelength was 606 nm. The average SMP excitation wavelength for the samples

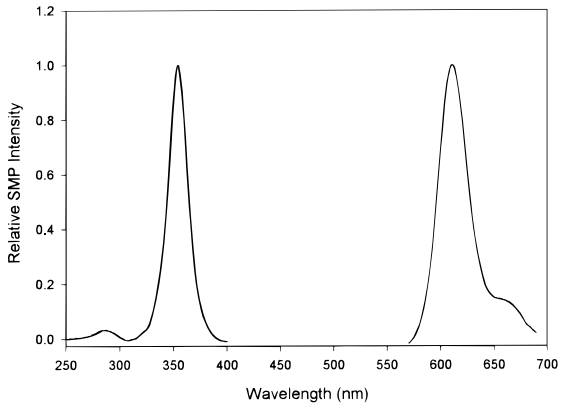

Figure 1. SMRTP excitation and emission spectra of 40 μg of a 0.005% modified DNA adduct sample adsorbed on 30% $TlNO_3$/NaOAc.

Table 1. Average SMP Excitation and Emission Wavelengths and Standard Deviations for BPDE−DNA Adducts and Tetrol I-1 Adsorbed on 30% $TlNO_3$/NaOAc and 1PS Paper

	excitation, nm	emission, nm
BPDE−DNA[a]	355 ± 2	611 ± 1[b]
Tetrol[a]	351 ± 1	606 ± 1
BPDE−DNA[c]	350 ± 1[d]	609 ± 1
Tetrol I-1[c]	346 ± 0.2	612 ± 1

[a] Data for 30% $TlNO_3$/NaOAc. [b] Average value and standard deviation for samples of BPDE−DNA adduct (40 μg) modified at the first four levels. [c] Data for 1PS paper. [d] Average value and standard deviation for samples of BPDE−DNA adduct (6, 12, 24, and 36 μg) modified at 1.0×10^{-3}, 5.0×10^{-4}, and 1.0×10^{-4}%.

of BPDE−DNA adducts (40 μg) was 355 nm. For tetrol I-1, the excitation wavelength was 351 nm. The excitation wavelength data obtained also showed that tetrols were not present in the BPDE−DNA samples. The average SMP excitation and emission wavelengths and standard deviations of these wavelengths are given in Table 1. It should be mentioned that the reproducibility of five measurements for the excitation wavelength of a europium chloride phosphorescence standard was ±0.1. Similar measurements for the phosphorescence emission wavelength for europium chloride showed that the wavelength did not vary. Thus, the luminescence instrument gave very good wavelength reproducibility. The wavelength data of the other DNA adduct samples adsorbed on 30% $TlNO_3$/NaOAc also showed that the samples did not contain tetrols. DNA adduct samples modified at 1.0×10^{-3}, 5.0×10^{-4}, and 1.0×10^{-4} % were adsorbed on 1PS paper at 6, 12, 24, and 36 μg, and their SMP excitation and emission spectra were compared with the SMP excitation and emission spectra of tetrol I-1 adsorbed in 1PS paper. The SMP emission spectra for the BPDE−DNA adducts and tetrol I-1 were somewhat difficult to distinguish from one another because of the similarity of their emission wavelengths (See Table 1). However, tetrol I-1 gave a major excitation band at 346 nm, whereas the BPDE−DNA adducts had an average excitation band for the three samples adsorbed at four different microgram amounts of 350 nm (Table 1). Thus, the excitation bands for tetrol I-1 and the BPDE−DNA adducts were used as a gauge to detect tetrols. For all the BPDE−DNA samples investigated, there was no indication of tetrols present in the DNA samples.

Several other precautions and experiments were carried out to ensure that tetrols were not present with the BPDE−DNA samples. All stock solutions of BPDE−DNA adducts were extracted with ether to remove tetrols. The sample preparation procedures for SMP were performed under very low light level in the laboratory. Previously, Tjioe et al.[26] showed by using solid-matrix fluorescence (SMF) that tetrols were not formed from BPDE−DNA adducts (modified at 1.9%) adsorbed on 1PS paper after exposure to 346-nm radiation from a 450 W lamp for 60 min. The 1PS paper inhibited the formation of tetrols.[26] In this work, blank 1PS paper, tetrol I-1 (40 ng) adsorbed on 1PS paper, and 8 μg BPDE−DNA adducts (modified at 5.0×10^{-4}%) adsorbed on 1PS paper were extracted with ethanol because tetrols are very soluble in ethanol. The ethanol extracts were evaporated to dryness and the residue was dissolved in 1 mL of ethanol, and then the solution fluorescence spectra were acquired. The fluorescence spectra from the extracts for the blank and BPDE−DNA adduct samples were identical, indicating that no tetrols were present in the BPDE−DNA adduct samples. The ethanol extract from the tetrol sample readily gave the fluorescence emission spectrum of tetrol. These results indicated that the tetrols were not present in BPDE−DNA adducts. In earlier work, Li and Hurtubise[30] confirmed that tetrols were not present in a BPDE−DNA adduct sample (modified at 2.6%) that was adsorbed on 30% $TlNO_3$/NaOAc and handled in the same fashion as the samples in this work. They extracted several samples of BPDE−DNA adduct that were adsorbed on 30% $TlNO_3$/NaOAc with ether or ethanol. The extracts were evaporated, and the residues were dissolved in 30% MeOH/H_2O. The solutions were tested via fluorescence for tetrols, and no tetrols were detected. It is clear from the experimental results and precautions taken that tetrols were not detectable in the BPDE−DNA samples investigated in this work.

Quantitative Aspects of SMP of the BPDE−DNA Adducts. It is obvious that a BPDE−DNA adduct sample modified at a given level would show an increase in SMP with an increase in sample size. Also, to determine the BPDE−DNA adduct content in the samples at very low levels of modification, relatively large sample sizes are needed. Thus, it was important to find the sample size range that gave a linear relationship between the relative SMP signals and the amounts of sample that were modified at the same level. At these very low percentages of modification in this work, we found that SMP versus sample size for the same percent modification (0.001%) gave good linearity to about 36 μg using Whatman 1PS filter papers (Figure 2). The plot in Figure 2 gave a linear correlation coefficient of 0.987 with a *y*-intercept of 0.081 and a maximum relative SMP signal of 1.0. Similar results were obtained for BPDE−DNA samples adsorbed on Whatman 1PS paper that were modified at the other levels. However, the samples modified at 5.0×10^{-5} % and 1.0×10^{-5} gave a linear range to 25 μg of an adsorbed adduct sample. Because of limited amounts of the modified DNA samples, only the DNA sample modified at 5.0×10^{-4}% was investigated for the change in SMP as a function of the amount of sample adsorbed on 30% $TlNO_3$/NaOAc. A plot of SMP versus sample size was linear to 80 μg of adsorbed sample. The linear correlation coefficient was 0.999 with a *y*-intercept of 0.008 and a maximum relative SMP signal of 1.0.

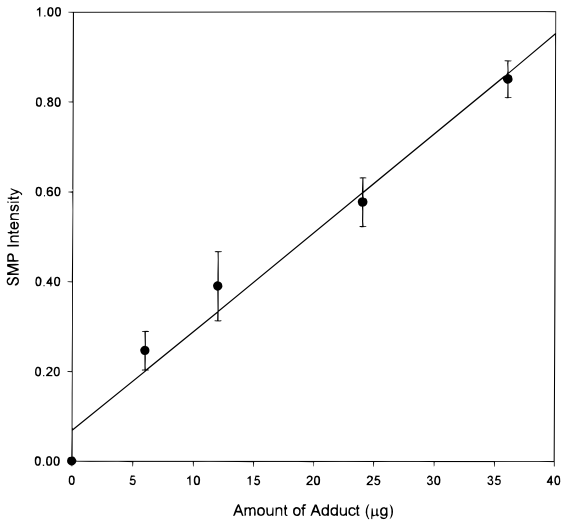

Figure 2. SMRTP versus sample size of the 0.001% modified DNA on Whatman 1PS filter paper with 25 μg $TlNO_3$.

To demonstrate that a linear relationship could be obtained between SMP and different percentages of modification, various plots of SMP versus percent modification were acquired for the modified DNA samples adsorbed on Whatman 1PS paper and 30% $TlNO_3$/NaOAc. A plot of SMP versus 36 μg each of 1.0×10^{-4}, 5.0×10^{-4}, 1.0×10^{-3}, and 5.0×10^{-3}% modification on Whatman 1PS paper gave a nonlinear relationship because the SMP signal for the 5.0×10^{-3}% sample was not linear with the SMP signals for the other three samples. This was most likely a result of the nonuniform excitation of the SMP of the sample modified at 5.0×10^{-3}%. However, a plot of SMP versus 36 μg each of 1.0×10^{-4}, 5.0×10^{-4}, and 1.0×10^{-3}% gave a good linear relationship with a correlation coefficient of 0.991. A similar plot of SMP versus 40 μg each of 1.0×10^{-4}, 5.0×10^{-4}, and 1.0×10^{-3}% modification on 30% $TlNO_3$/NaOAc gave an excellent linear relationship with a correlation coefficient of 0.999. It should be mentioned that the SMP signals were corrected with the appropriate blank signals from unmodified DNA samples (36 μg of DNA with 1PS paper and 40 μg of DNA with 30% $TlNO_3$/NaOAc). Figure 3 shows the results obtained for the samples modified at 1.0×10^{-4}, 5.0×10^{-4}, and 1.0×10^{-3} % on 30% $TlNO_3$/NaOAc. When an attempt was made to obtain a linear relationship between SMP and percent modification of DNA samples modified with the two different batches of DNA, linearity was not obtained. The samples modified by 5.0×10^{-4}, 1.0×10^{-4}, 5.0×10^{-5}, and 1.0×10^{-5}% were used in the experiments. The first two samples were modified with the first batch of purified DNA, and the latter two samples were modified with the second batch of DNA. These results show that it would be important to use a "standardized" DNA sample for day-to-day determination of the BPDE adduct content in DNA samples. Use of such standards and other similar comparative approaches have been initiated for BPDE−DNA adduct monitoring by immunoassays, synchronous fluorescence spectrometry, and ^{32}P post-labeling.[8,11,33,34]

The SMP relative standard deviations for triplicate runs for the BPDE−DNA adduct samples (36 μg) on Whatman 1PS paper were 4.8, 6.9, and 26% for the DNA samples modified by $1.0 \times$

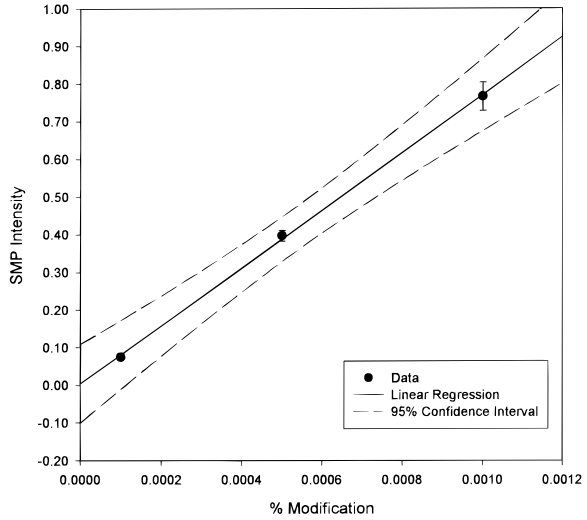

Figure 3. SMRTP of 40 μg of 0.001, 0.0005, and 0.0001% modified DNA adduct samples on 30% $TlNO_3$/NaOAc powder matrix.

Table 2. Detection Limit for Benzo[*a*]pyrene−DNA Adducts[a,b]

matrix	% modification	fmol/μg	adduct/nucleotides
1 PS filter paper	2×10^{-5}	0.6	$2/1 \times 10^7$
30% $TlNO_3$/NaOAc	2×10^{-5}	0.6	$2/1 \times 10^7$

[a] The detection limits were calculated as the % modification that gave 3 times the standard deviation of the blank divided by the slope of the calibration curve. [b] A DNA sample modified at 1.0×10^{-3}% is the same as 1 adduct in 10^5 nucleotides which is equivalent to 30 fmol of BPDE per microgram of DNA.

10^{-3}, 5.0×10^{-4}, and 1.0×10^{-4} %, respectively. The SMP relative standard deviations for triplicate runs for the same BPDE−DNA adduct samples (40 μg) on 30% $TlNO_3$/NaOAc were 4.8, 5.1, and 16%, respectively. Table 2 compares the detection limits that were obtained with Whatman 1PS filter paper and 30% $TlNO_3$/NaOAc. As Table 2 shows, the detection limit with Whatman 1PS filter paper was the same as that with 30% $TlNO_3$/NaOAc. Samples of DNA modified by 5.0×10^{-5} and 1.0×10^{-5}% gave SMP signals, but as discussed in the preceding paragraph, these samples were modified with a different batch of DNA. Thus, limits of detection with these samples were not calculated. However, the calculated adduct per nucleotide for these samples was $1/(2.0 \times 10^6)$ and $1/(1.0 \times 10^7)$, respectively.

CONCLUSIONS

In conclusion, we have demonstrated for the first time that linear and reproducible SMP signals can be obtained between SMP and the percentages of DNA modified by BPDE. Thus, this approach can be used for the direct determination of BPDE−DNA adducts. For example, it has been reported that one adduct in 10^4−10^5 bases results from cell tissue culture exposed to BPDE.[35] As indicated in Table 2, the detection limits by SMP are much lower than the levels found in cell tissue culture. Also, the detection limits are in the range of those of some human samples. Weston and Bowen[8] have reported 1.2 fmol of BPDE per microgram of DNA in a human lung sample. In addition, Shields et al.[33] indicated that a human lung sample contained 0.46 fmol per microgram ofDNA. It should be emphasized that the BPDE−DNA adduct samples modified at 5.0×10^{-4}% and adsorbed on 30% $TlNO_3$/NaOAc gave SMP signals that were linear to 80 μg. Because of limited amounts of sample, higher masses of BPDE−DNA were not tested with the 30%$TlNO_3$/NaOAc. The limits of detection in Table 2 were acquired with 40-μg samples of BPDE−DNA. However, if samples of 80 μg of BPDE−DNA were used to calculate the limits of detection, the detection limits would be decreased by a factor of approximately 2.

The amounts of DNA needed for the determination of BPDE−DNA adducts is an important consideration. For example, in one method at least 1 mg of DNA was needed for the determination of BPDE−DNA adducts in human lung samples. For this method, enzymatic digests were applied to immunoaffinity columns, and the fractions were hydrolyzed to tetrols. Then, the fluorescence of the tetrols was detected.[8] In another method for the BPDE−DNA adducts in human samples, 50−150 μg of DNA was used. For this method, tetrols were isolated by high-performance liquid chromatography and then detected by fluorescence spectrometry.[10] With 1PS paper, 36 μg of modified DNA was used, and 40 μg of the modified DNA was employed with 30% $TlNO_3$/NaOAc. Thus, the BPDE−DNA sample sizes used in this work were lower than the DNA samples sizes used for the human DNA samples.[8,10]

As discussed earlier, SMP was readily detected from a batch of DNA that was modified at 5.0×10^{-5} and 1.0×10^{-5}%. However, these samples were not used to calculate limits of detection because of the need to develop a standard approach for relating SMP intensity to percent modification when different batches of DNA are used in the modification procedure. The sample modified at 1.0×10^{-5}% represents one adduct in 10^7 bases. These results indicate that under the appropriate conditions SMP could detect at least 1 adduct in 10^7 bases. Inexpensive instrumentation was employed in this work, and the methodology is simple. The detection limits are in the range of those found in some human samples.

ACKNOWLEDGMENT

This research project was supported by the U.S. Environmental Protection Agency Grant No. R824100. The helpful comments of Nicholas Geacintov are greatly appreciated.

Received for review May 21, 1999. Accepted August 3, 1999.

AC9905726

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