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Detection of Polycyclic Aromatic Hydrocarbon–DNA Adducts in Human Lung

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Synchronous fluorescence spectroscopy has been combined with immunoaffinity chromatography (IAC) and HPLC to detect polycyclic aromatic hydrocarbon (PAH)–DNA adducts and measure *r*-7,*t*-8-dihydroxy-*t*-9, 10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE)–DNA adducts in human tissues and cells. A monoclonal antibody (8E11) that recognizes a range of PAH–DNA adducts, but not chemically unrelated adducts, was used to prepare IAC columns. Samples of DNA (25 from human lung and 8 positive and negative controls) were hydrolyzed enzymically and subjected to IAC. Adducts captured by the antibodies and eluted in NaOH (50 mM) were analyzed for fluorescent properties. The spectral fluorescence excitation-emission matrices suggested the presence of mixtures of PAH–DNA adducts in some of the eluates. The eluates were subsequently hydrolyzed with acid (HCl, 0.1 N, 3 hr) and reanalyzed by synchronous fluorescence spectroscopy using a wavelength differential of 34 nm. In 6 of the 25 human lung DNA samples, materials with HPLC retention times identical to benzo[*a*]pyrene-7,10/8,9-tetrahydrotetrol were found to have fluorescence characteristics indistinguishable from pyrene. Comparisons with appropriate standards indicated that BPDE–DNA adduct levels were between 1 and 40 adducts in 10^8 unmodified nucleotides. No correlation was observed between lung DNA-adduct levels and measures of recent smoking (serum cotinine), but tissue samples taken from different portions of the same lungs showed variation in the DNA adduct levels detected. This finding complicates interpretation of the data and has important implications for the design of future experiments.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) form a major class of environmental pollutants; many of these compounds can be metabolized and activated to carcinogenic species that bind covalently with DNA (*1*). To measure genotoxic damage from PAHs, fluorescence techniques have been developed for the detection of DNA adducts, and *r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE)–DNA adducts have been identified in human tissues (*2*). Our strategy for detection and chemical characterization of adducts is based on combining the following preparative and analytical procedures: immunoaffinity chromatography is used to concentrate adducts of a specific chemical class (PAHs); HPLC facilitates separation of DNA hydrolysis products; and synchronous fluorescence spectroscopy (SFS), second derivative synchronous fluorescence spectroscopy, and the generation of fluorescence excitation-emission matrices (FEEMs) provide chemically specific detection systems. Using this strategy (*3,4*), levels of BPDE–DNA adducts in the range of 1–40 in 10^8 nucleotides have been measured in human lung.

Materials and Methods

Human lungs were collected at autopsy from the Department of Anatomic Pathology, University of Maryland. The tissues were stored at −70°C until DNA was extracted according to a previously described methodology (*5*). Enzymic digests of DNA were applied to immunoaffinity columns containing monoclonal antibodies directed against BPDE-modified guanosine (*6*). The columns were then washed extensively with buffer, and bound materials were eluted with NaOH. Fractions eluted in NaOH were further hydrolyzed with HCl, and FEEMs were generated in the synchronous mode. Products of the acid hydrolysis were then separated by HPLC. Materials with retention times equal to those of benzo[*a*]pyrene-7,10/8,9-tetrahydrotetrol were further subjected to SFS using a wavelength difference ($\Delta\lambda$) of 34 nm. Serum was obtained from donors and assayed for the presence of cotinine using a radioimmunoassay (*7*).

Results and Discussion

Synchronous fluorescence spectroscopy is highly specific for identification of aromatic nuclei because characteristic fluo-

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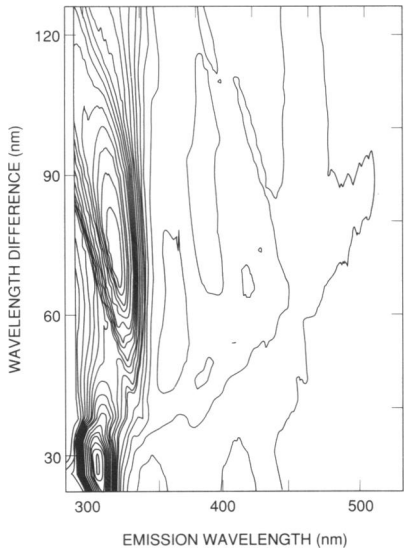

FIGURE 1. Contour plots showing fluorescence spectral excitation-emission matrices for extracts of human peripheral lung DNA (sample no. 1069).

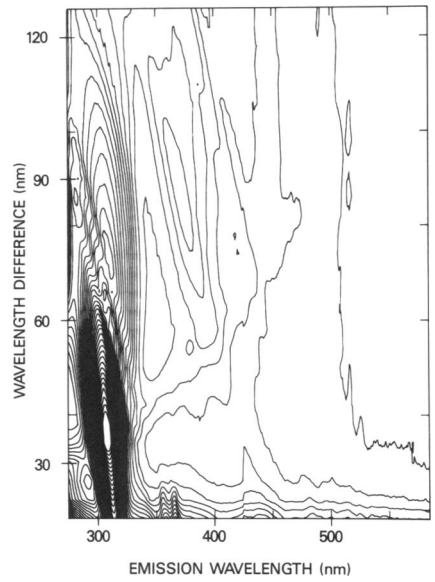

FIGURE 2. Contour plots showing fluorescence spectral excitation-emission matrices for extracts of human peripheral lung DNA (sample no. 1177).

rescence emissions can be detected when the distance between the excitation and emission monochromators is equal to the Stokes-shift of a given chemical compound (*8*). This is particularly useful in the case of pyrene because the Stokes-shift is large (28 nm). In addition, generation of FEEMs can demonstrate the presence of previously uncharacterized materials in complex mixtures. Figures 1 and 2 show FEEMs generated by SFS for materials isolated from human lung DNA by immunoaffinity chromatography (IAC) using the 8E11 antibodies. As these FEEMs are displayed as contour plots, the major features are indicated by fluorescence emission peaks in the region 290–350 nm for a range of Δλ values between 40 and 100 nm.

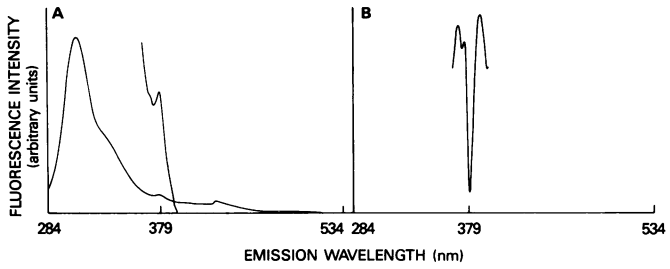

FIGURE 3. DNA samples were hydrolyzed with micrococcal nuclease. Adducts were concentrated by immunoaffinity chromatography (antibody 8E11) then hydrolyzed (0.1 N HCl) to tetrahydrotetrols. Organic solvent extracts of the acid hydroysates were subjected to HPLC, and materials with retention times identical to benzo[*a*]pyrene-7,10/8,9-tetrahydrotetrol were analyzed by synchronous fluorescence spectroscopy. (*A*) Zero-order synchronous fluorescence spectrum generated for an extract of human lung DNA (125 fmole isolated from sample no. 1132) (1 mg). (*B*) Corresponding second derivative spectrum.

The complexity of these matrices suggests the presence of multiple aromatic adducts in lung samples. The presence of the pyrene fluorophore (indicating BPDE–DNA adducts in the original DNA sample) is shown in Figure 1 by a fluorescence emission peak at 379 nm (Δλ 34 nm); however, this signal does not dominate the matrix. In these studies, specificity is obtained through the chromatographic procedures and selection of the fluorescence parameters. Specificity is confirmed by the quality of the spectra observed. Fluorescent emissions from the pyrene fluorophore are not always obvious upon FEEM analysis of a complex mixture, as shown in Figure 2. However, HPLC separation of the acid hydrolysate from which this FEEM was generated revealed a clear signal, specific for pyrene, when appropriate fractions were analyzed by SFS using Δλ 34 nm.

Using both SFS Δλ 34 nm and second derivative spectroscopy to analyze HPLC eluates of the hydrolysis products of IAC-concentrated adducts, the levels of BPDE–DNA adducts were determined for 25 lung samples. The zero-order and second-derivative synchronous fluorescence spectra (Δλ 34 nm) for materials isolated from human lung by IAC and HPLC are shown in Figure 3. The presence of specific spectral signals (379 nm, emission λ) and absence of other nonspecific signals indicate that the isolate is pure. In previous studies the identity of this material as a pure isolate of the benzo[*a*]pyrene-7,10/8,9-tetrahydrotetrol was confirmed by GC–MS (*4,9*).

The levels of BPDE–DNA adducts detected in human peripheral lung samples (Table 1) were determined by comparison with a standard curve that was prepared from fluorescence peak heights generated by an authentic tetrol standard and radiolabeled positive control samples that were assayed together with human samples to account for efficiency of the preparative procedure (Table 1). Tetrol recovery from ^{3}H-BPDE-modified DNA samples ranged from 26 to 66% for the whole procedure. Assays were performed on coded sample sets that consisted of 4–20 human DNA samples and at least 2 positive and 2 negative control samples. Human peripheral lung DNA was assayed from a total of 25 individuals. There was sufficient tissue to independently extract DNA from 12 of the lung samples twice and 4 of them 3 times. Lung DNA from six individuals contained BPDE–DNA adduct levels between 23 and 1215 amole/

Table 1. Levels of serum cotinine, demographic data, and benzo[a]pyrene (BaP) adduct levels in lungs of autopsy donors from the Baltimore, Maryland, area of the United States.

Case no.	A/G/R[a]	Cotinine, ng/mL	BaP adducts[b] I	II	III
1069	44/M/W	414	75	151	36
1132	24/M/W	SNC[c]	125	289	ND[d]
1128	45/F/B	245	29	71	
1177	21/M/W	52	35	1215	
1119	42/M/B	SNC	23		
1143	17/M/B	ND	35		
Controls, %[c]			42-66	26-40	42-63

[a]Age/gender/race (age in years, M, male; F, female, B, black; W, white).
[b]Attomole benzo[a]pyrene-diol epoxide (BPDE)/μg DNA recovered as BaP-tetrol determined on three separate occasions. (I, II, and III represent materials assayed from different portions of the same lung.)
[c]SNC, serum not collected.
[d]ND, not detected; below the limit of detection (6 pg BaP-tetrol/mL; 2 μg cotinine/mL).
[c]Percent recovery of 250-700 amole/μg BPDE-DNA.

μg DNA (Table 1). Variation in the BPDE-DNA values obtained for some of the human samples is evident (samples 1132 and 1177); however, a similar variability was not observed for the positive control materials (Table 1). Replicate DNA samples were prepared from stored, frozen lung at different times; therefore these data suggest heterogeneity of adduct levels in different pieces of the same lung. No statistical correlation between serum cotinine levels and BPDE-DNA adduct levels was observed in this small sample set.

These data show that mixtures of PAH-DNA adducts can be isolated from human DNA and can be detected by SFS/FEEM. This is because the antibodies used for IAC are specific for PAH-DNA adducts, and SFS/FEEM yields characteristic information on aromatic nuclei. Thus far, BPDE-DNA adducts have been shown to be present in adduct mixtures isolated from human lung. These studies are being extended in four major directions: detection of PAH-DNA adducts by SFS/FEEM may provide a screening technique for as yet unknown components of complex mixture samples; in combination with HPLC, attempts are being made to identify PAH-DNA adducts other than BPDE; other tissues are being considered for the same adduct; the fluorescence techniques described here, in combination with IAC and HPLC, may provide corroboration for less specific techniques (enzyme immunoassays and the ^{32}P-postlabeling assay).

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REFERENCES

1. Osborne, M. R., and Crosby, N. T. Benzopyrenes. In: Cambridge Monographs on Cancer Research (M. R. Osborne and N. T. Crosby, Eds.), Cambridge University Press, New York, 1987, pp. 73-164.
2. Weston, A., Bowman, E. D., Manchester, D. K., and Harris, C. C. Fluorescence detection of lesions in DNA. In: DNA Damage and Repair in Human Tissues (B. M. Sutherland and A. D. Woodhead, Eds.), Plenum Press, New York, 1990, pp. 63-81.
3. Manchester, D. K., Weston, A., Choi, J. S., Trivers, G. E., Fennessey, P., Quintana, E., Farmer, P. B., Mann, D. L., and Harris, C. C. Detection of benzo[a]pyrene diol epoxide-DNA adducts in human placenta. Proc. Natl. Acad. Sci. U.S.A. 85: 9243-9247 (1988).
4. Weston, A., and Bowman, E. D. Fluorescence detection of benzo[a]pyrene-DNA adducts in human lung. Carcinogenesis 12: 1445-1449 (1991).
5. Weston, A., Willey, J. C., Modali, R., Sugimura, H., McDowell, E. M., Resau, J., Light, B., Haugen, A., Mann, D. L., Trump, B. F., and Harris, C. C. Differential DNA sequence deletions from chromosomes 3, 11, 13 and 17 in squamous cell carcinoma, large cell carcinoma and adenocarcinoma of the human lung. Proc. Natl. Acad. Sci. U.S.A. 86: 5099-5103 (1989).
6. Santella, R. M., Lin, C. D., Cleaveland, W. L., and Weinstein, I. B. Monoclonal antibodies to DNA modified by a benzo[a]pyrene diol epoxide. Carcinogenesis 5: 373-377 (1984).
7. Haley, N. J., Hoffmann, D., and Wynder, E. L. Uptake of tobacco smoke components. Banbury Rep. 23: 3-19 (1986).
8. Vo-Dinh, T. Synchronous luminescence spectroscopy: methodology and applicability. Appl. Spectr. 36: 576-581 (1982).
9. Weston, A., Manchester, D. K., Poirier, M. C., Choi, J. S., Trivers, G. E., Mann, D. L., and Harris, C. C. Derivative fluorescence spectral analysis of polycyclic aromatic hydrocarbon-DNA adducts in human placenta. Chem. Res. Toxicol. 2: 104-108 (1989).