change over a 48-h period, while the sample exposed to the moisture in room air increased significantly in -OH concentration. In another set of experiments samples of $SiCl_4$ initially containing 15 ppm of -OH were stored in tightly closed Teflon bottles for 10 days in dry N_2 and in room air. The $SiCl_4$ stored in N_2 was unchanged, while that stored in air increased to 45 ppm of -OH. Clearly $SiCl_4$ even in Teflon bottles will hydrolyze unless stored in a dry atmosphere. Despite their porosity to moisture, however, Teflon bottles are useful because they effectively prevent volatilization of $SiCl_4$.

ACKNOWLEDGMENT

We gratefully acknowledge the assistance of W. G. French, S. S. DeBala, G. A. Pasteur, J. E. Kessler, J. B. MacChesney, A. D. Pearson, and S. R. Nagel.

Registry No. $SiCl_4$, 10026-04-7; HCl, 7647-01-0; $SiHCl_3$, 10025-78-2; Si_2OCl_6, 14986-21-1; $CHCl_3$, 67-66-3; $CH_3(CH_2)_4CH_3$, 110-54-3; $Ph_3(OH)Si$, 791-31-1.

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RECEIVED for review September 29, 1986. Accepted December 19, 1986.

Laser-Induced Room-Temperature Phosphorescence Detection of Benzo[*a*]pyrene–DNA Adducts

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The room-temperature phosphorescence (RTP) spectrum of benzo[*a*]pyrene-*r*-7,*t*-8,9,10-tetrahydrotetrol (BP-tetrol) has been measured using laser excitation. The BP-terol was obtained by acid hydrolysis of the *r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE)–DNA adducts. BPDE is the ultimate carcinogenic metabolite of benzo[*a*]pyrene (BP). The BP-tetrol sample was measured on a filter paper substrate pretreated with a heavy-atom salt, thallium acetate, used to increase the phosphorescence signal of BP-tetrol. The detection limit of BPDE in in vitro modified BPDE–DNA was about 15 fmol. The results indicate the RTP would be useful as a simple and practical screening tool for monitoring BPDE–DNA adducts and related BP metabolites in biological samples.

The mutagenic and carcinogenic activity of many polynuclear aromatic (PNA) compounds (*1*) has been the focal point for concern about human exposure to these species in the workplace and in residential environments. Polynuclear aromatic compounds, which are products of incomplete combustion of organic materials, are widely distributed in the environment. Since combustion processes occur frequently in many industries, PNA compounds have been found in a large number of workplace environments (*2–4*). Residential activities, including cooking, woodstove burning, and cigarette smoking, are indoor emission sources of PNA pollutants (*5*). An important PNA compound of great interest to toxicologists and cancer researchers is benzo[*a*]pyrene (BP). Studies have shown that BP is metabolically activated to electrophilic intermediates, which bind convalently to DNA (*3*). A specific diol epoxide derivative of BP, *r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) was found as the major carcinogenic metabolite involved in binding to DNA (*6*). Metabolized BP is eliminated through the urine and feces (*7*). Since the carcinogenic activity of a compound could be associated with the degree to which it binds to DNA, there has been a great deal of interest in analytical techniques that are capable of detecting DNA–carcinogen interactions and monitoring human exposure to PNA compounds.

Several techniques, including mass spectrometry (*8*), synchronous fluorescence (*9, 10, 20*), liquid chromatography (*11, 12*), fluorescence line narrowing spectroscopy (*13*), ^{32}P-postlabeling autoradiography (*14*), and enzyme-linked immunoassay (*15*), have been developed to detect carcinogen–DNA

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adducts. This communication describes a new procedure based on a phosphorescence technique at room temperature to detect BPDE–DNA adducts and BP metabolites adsorbed on filter paper. The room-temperature phosphorescence (RTP) technique is a relatively new approach in phosphorescence analysis. Unlike conventional low-temperature phosphorimetry, the RTP technique does not require cryogenic equipment and low-temperature refrigerant. The basic principle of RTP has been described elsewhere (*16*). Only the main features underlying the RTP process and methodology are presented here. Phosphorescence is an emission from the lowest excited triplet state to the singlet ground state. Due to its spin-forbidden nature, phosphorescence emission exhibits typically longer decay times (milliseconds to several seconds lifetimes) than the spin-allowed fluorescence process (10^{-10} to 10^{-7} s lifetime). In liquid solutions at room temperature, bimolecular and intramolecular quenching processes usually cause radiationless deactivation of the triplet state (*17*). Oxygen, an efficient triplet quencher, is also a major contributor to the radiationless decay of the triplet state. Due to these radiationless deactivation processes, phosphorescence can normally be observed only when the solution is frozen into rigid matrices. Conventional methods in phosphorimetry, therefore, involve careful preparation of oxygen-free solutions, or insertion of the compounds of interest into polymer samples, or use of rigid matrices of frozen organic solvents. While the first two techniques involve tedious and time-consuming sample preparation, the last method requires low-temperature experiments and cryogenic equipment. Under certain conditions, phosphorescence at room temperature can be observed from various PNA compounds adsorbed on solid substrates, such as silica, alumina, paper, and asbestos. This type of phosphorescence is assumed to originate from surface adsorbed molecules, since none could be observed from finely ground samples of free crystalline PNA compounds. The presence of heavy atoms in the immediate adjacent environment of the molecule can significantly enhance the population of the triplet state and, therefore, the phosphorescence intensity. This phenomenon is known as the external heavy-atom effect, a quantum mechanical process that increases the molecular spin–orbit coupling leading to an increase of the phosphorescent triplet state of the molecules. The method has not been used for the detection of metabolites of these PNA species. In this study we have investigated whether it is possible to detect the RTP signal of biological systems derived from metabolic activation of PNA compounds. The results indicate that the RTP technique provides a promising screening method for monitoring human exposure to carcinogenic PNA compounds.

EXPERIMENTAL SECTION

The RTP experimental procedure method involves four steps including (a) substrate preparation (optional pretreatment with heavy-atom salts), (b) sample delivery, (c) drying, and (d) spectroscopic measurement. The entire experimental procedure takes only 10 min.

Our own procedure involves cutting filter paper into 6-mm-diameter disks with a standard office hole punch. Aliquots of solution (2.5 μL) were then spotted on the paper circles by use of microsyringes. Since moisture can quench the RTP emission, predrying was achieved by heating the samples with infrared heating lamps. Continued drying during the measurement was accomplished by blowing warm and dry air through the sample compartment. Phosphorimetric measurements were conducted with a commercial Perkin-Elmer spectrofluorometer (Model 43A) equipped with a rotating phosphoroscope. In this work, the filter paper substrate (Whatman 42) was pretreated with the heavy-atom salt thallium acetate (1 M in ethanol–water) before sample solution delivery.

The BPDE–DNA adducts were prepared by adding 50 μL of a solution of 3 mM BPDE (in MeOH) to 1 mL of DNA (1.2 mg/mL) dissolved in H_2O and incubating overnight at 37 °C. The resulting solution was passed through a Sep-PAK C-18 (Waters Corp., Milford MA) cartridge which was preequilibrated in H_2O. The cartridge was washed with 5 mL of H_2O and the aqueous solutions were pooled to give an adduct preparation that was 8.6×10^{-6} M in pyrenyl residues. This value was calculated from the absorbance at 343 nm and the extinction coefficient of 49 000 M^{-1} cm^2. The purified DNA adduct did not have any detectable free BP-tetrol when it was recycled through a fresh Sep-PAK cartridge. Acid hydrolysis of BPDE–DNA adducts at 90 °C for 90 min gave rise to an isomeric mixture of BP-tetrols, where the major component was the 7-*r*,8-*t*,9-*t*,10*c* configuration (often referred to as BPT-I).

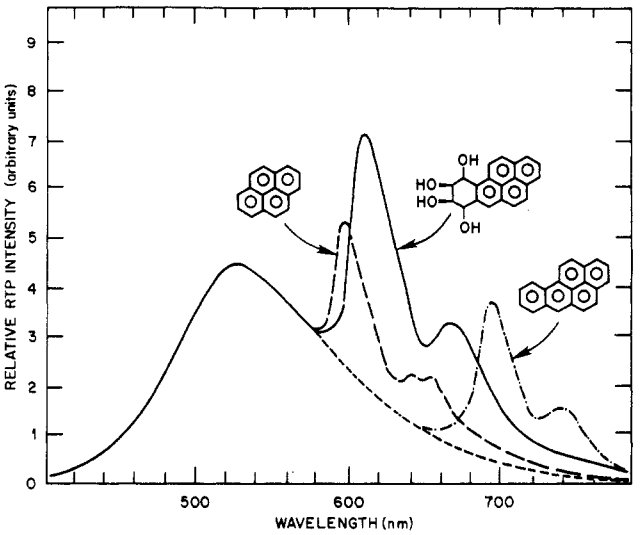

Figure 1. RTP spectrum of BPT obtained by hydrolysis of BPDE–DNA adducts. For comparison the RTP spectra of pyrene and BP were also shown.

RESULTS AND DISCUSSION

Figure 1 shows the RTP spectrum of BPT spotted on Whatman 42 filter paper treated with thallium acetate (1 M in ethanol/water solvent, 1/1, (v/v)). A helium cadmium continuous wave (CW) laser emission at 325 nm was used as the excitation source. The BPT species were obtained by acid hydrolysis of BPDE–DNA adducts (*18*). As shown in this figure, the BPT species can be detected by RTP and characterized by their two phosphorescence bands at 618 and 668 nm. We have also confirmed the RTP spectrum of BPT species formed by acid hydrolysis by comparing the RTP spectrum from the hydrolysate of BPDE with that of BPT–I obtained through the National Cancer Institute Chemical Carcinogen Repository. For comparative purposes, the RTP spectra of pyrene and BP are also depicted in Figure 1. Although BPT is a metabolite derived from BP and contains a pyrenyl system, its RTP spectrum is markedly different from those of pyrene and BP. The most intense RTP band of BPT at 618 nm can be easily differentiated from the main band of pyrene at 598 nm and from that of BP at 700 nm.

The external heavy-atom effect, which is a very useful tool to improve the sensitivity of the RTP method, is illustrated in Figure 2. Great care, however, has to be taken to apply this technique to complex biological systems. Since the external heavy-atom effect is most efficient when the heavy atoms are close to the analyte molecules, the proximity of the heavy atoms and the analyte molecules is an important factor. Direct RTP measurements of the BPDE–DNA adducts did not produce strong RTP signals even in the presence of thallium acetate (Figure 2, curve b). The decreased efficiency of the heavy-atom effect on the BPDE bound to the DNA adducts might be due to the close proximity of the DNA bases and possible steric hindrances for optimal BPT–metal ion

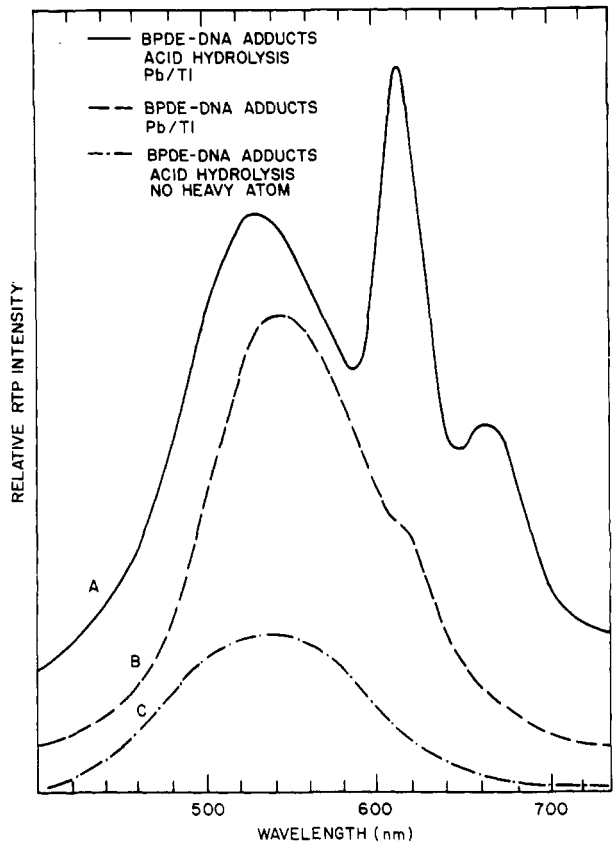

Figure 2. RTP spectra of BPT (curve A), BPDE–DNA adducts (curve B), and paper substrate (curve C). Thallium acetate was used as the heavy-atom perturber.

interaction. This does not present a major limitation since the BPT portions can be released by the relatively simple and previously described acid hydrolysis procedure. Figure 2 shows the comparative results of RTP measurements of BPDE–DNA adducts and of BPT. The data showed that the RTP intensity of BPT on paper treated with thallium acetate increased by a factor $\geq 10^3$ over the signal obtained with the untreated substrate. Without the heavy-atom effect, the phosphorescence signal of BPT on Whatman 42 paper substrate was extremely weak and undistinguishable from the paper background (curve c).

We have conducted RTP measurements of BPT using different heavy-atom salts to determine the most efficient heavy-atom system. Previous investigations of the heavy atom effect on a variety of PNA compounds have shown that both sensitivity and selectivity can be improved by the use of different heavy-atom systems (*19*). In addition to thallium acetate, we evaluated the use of lead acetate, silver nitrate, sodium bromide, and sodium iodide. Among all the heavy-atom salts investigated, thallium acetate appeared to produce the strongest phosphorescence enhancement. It is noteworthy that the RTP procedure can directly detect the BPT portions released from the BPDE–DNA adducts in the complex mixture. No elaborate chromatographic separation was required to separate BPT from the DNA bases. Selectivity of detection for BPT in the complex mixture was possible because the laser excitation at 315 nm was absorbed solely by BPT. The DNA bases, which absorb at lower wavelength ranges, were not excited by the laser radiation.

A main advantage of the RTP technique is the small amount of sample required. Since only a 2.5-μL sample solution is required, the absolute amount of BPT measured in Figure 1 was only 15 pmol. The detection limit of BPT using thallium acetate as the heavy-atom salt was about 15 fmol. Considering that BPT is usually extracted from about 50 μg of BPDE–DNA adduct hydrolysate, this procedure can detect about 2 adducts per 10^7 nucleotides. The sensitivity of the RTP technique is comparable to other spectrometric methods (*9, 10, 13*) but inferior to that of ^{32}P-postlabeling autoradiography (*14*), which requires the use of radioactive ^{32}P labels. The combined use of larger quantities of DNA (5 mg), which requires a larger amount of tissue, coupled with Sep-PAK concentration of the released tetrols, would lead to a detection sensitivity of about 1 adduct in 10^9 bases.

CONCLUSION

The results of this study have demonstrated that RTP can be used to detect trace levels of BPT species derived from BPDE–DNA adducts following an acid hydrolysis procedure. Although the spectral selectivity of RTP spectra is less than that of high-resolution low-temperature spectra, the method is characterized by its simplicity and does not require sophisticated and expensive instrumentation and cryogenic refrigerant. Thus it is well suited for routine analysis and screening procedure. Other advantages of this technique are the absence of extensive cleanup procedures and a reasonably good sensitivity. The RTP approach could provide an additional practical tool to monitor human exposure to carcinogenic and mutagenic PNA species.

ACKNOWLEDGMENT

The authors acknowledge the assistance of R. J. Ward and G. H. Miller in the preparation and preliminary measurements of the BPDE–DNA adduct samples.

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RECEIVED for review September 29, 1986. Accepted December 29, 1986. This research was jointly sponsored by the U.S Environmental Protection Agency (Interagency Agreement DW89931767-01-0) and the Office of Health and Environmental Research, U.S. Department of Energy under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems.