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TIME-DEPENDENT BLUE SHIFT OF THE FLUORESCENCE ORIGIN BAND OF BENZO[*a*]PYRENE (BP)-DERIVED BP-6-N7ADE ADDUCTS IN GLASSES

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Abstract We report unusual spectroscopic properties of the benzo-[*a*]pyrene (BP)-derived one-electron oxidation DNA adduct, BP-6-N7Ade, in glasses at low temperatures. Time-resolved fluorescence emission and fluorescence excitation spectra showed that the position of the origin bands shift to higher energies with longer delay time of the observation window. A non-linear and time-dependent Stokes shift has been revealed. This effect has not been observed for any BP-derived diol-epoxide adduct. Such a blue-shift, in opposite direction to the shift expected from solvation dynamics, has not been previously reported. The experimental data are interpreted in terms of a distribution of conformers and a matrix-induced frequency dependent dipole moment change across the inhomogeneously broadened absorption band.

Keywords: benzo[*a*]pyrene; one-electron oxidation adduct; BP-6-N7Ade adduct; fluorescence spectroscopy; Stokes shift.

INTRODUCTION

Benzo[*a*]pyrene (BP), one of the most carcinogenic polycyclic aromatic hydrocarbons (PAH) presently known,[1] forms two types of adducts: stable adducts that remain covalently bound to DNA under normal conditions of isolation, and depurinating adducts that are lost from DNA. One pathway involves the metabolic activation to diol-

epoxide (DE) intermediates; in this case both stable [1,2] and depurinating adducts are formed [2]. BP, when activated via a one-electron oxidation process, binds covalently to the N7 position of adenine or to the N7 or C8 positions of guanine residues [2,3]. Many stable and depurinating BP-derived DNA adducts have been identified in *in vitro* and *in vivo* experiments [1-3].

Low-temperature, time-resolved, fluorescence spectroscopy has proven to be a valuable tool for characterization of both stable and depurinating DNA adducts from various PAHs [2-4]. Fluorescence line-narrowing spectroscopy (FLNS) has been shown to be capable of distinguishing between a given PAH metabolite covalently bound to different DNA bases and to different nucleophilic centers of a given base [4]. FLNS interfaced with capillary electrophoresis (CE-FLNS) provides a valuable on-line technique for characterizing analytes in complex mixtures[5].

In this report we will briefly describe the results of a spectroscopic and theoretical investigation of the BP-6-N7Ade adduct. Time-resolved fluorescence spectra revealed that, in contrast to the BPDE-10-N7Ade type adducts the (0,0) band of BP-6-N7Ade shifts blue as a function of the delay time of the observation window. Since such behavior has not been observed before, a detailed spectroscopic study of this phenomenon was initiated. Quantum mechanical calculations of the change in the dipole moment upon excitation of the BP-6-N7Ade adduct were used to guide the interpretation of the low-temperature fluorescence spectra.

MATERIALS AND METHODS

Sample Preparation

Molecular structures of the BPDE-10-N7Ade and BP-6-N7Ade adducts are shown in the inserts of Figure 1, frames A and B, respectively. Both adducts were provided by Dr. E. Cavalieri (Eppley Institute for Research in Cancer, Omaha, NE). Proof of structures was obtained by means of NMR and tandem mass spectrometry [6]. The purity of both adducts was established via CE-FLNS. Adduct concentrations of all samples were $\sim 10^{-6}$ M in ethanol.

Low Temperature Fluorescence Spectroscopy

High-resolution FLN spectra ($S_1 \leftarrow S_0$ excitation; T = 4.2 K) and low-resolution fluorescence spectra ($S_2 \leftarrow S_0$ excitation; T = 77 K) were recorded using as excitation source a Lambda Physik FL-2002 dye laser pumped by a Lambda Physik Lextra 100 XeCl excimer laser. Low-resolution spectra were obtained using non-selective excitation at 308 nm. Samples were cooled in a glass cryostat with quartz optical windows. Fluorescence was dispersed by a McPherson 2061 1-m focal length monochromator, and detected by a Princeton Instruments IRY 1024/G/B intensified photodiode array. For time-resolved detection a Princeton Instruments FG-100 pulse generator was employed; various detector delay times and gate widths were utilized.

Calculations

Structural optimization of the BP-6-N7Ade adduct was carried out utilizing methods of molecular mechanics (MM). Calculations were performed with HyperChem's molecular modeling program. We have employed the MM+ force field parameters developed for organic

molecules. The Polak-Ribiere method was used for molecular mechanics optimization. In order to calculate thermodynamically favored conformations, separated from MM structures by energy barriers, we used quenched dynamics (i.e. simulated annealing) to explore the conformational space. Each of the structures was minimized and then subjected to 50 ps of molecular dynamics at various temperatures between 300 and 400 K. Starting and final temperatures in a dynamic run were set to 0 K, and the heat and cool times were set to 5 ps; the step size was 0.0005 ps. Simulations for the BP-6-N7Ade adduct were performed *in vacuo* and in a periodic box with 452 water molecules equilibrated at 300 K and 1 atmosphere to solvate a molecular system. Adducts were optimized using a semi-empirical (PM3) method.

RESULTS AND DISCUSSION

Comparison of the normalized fluorescence origin bands of the two BP-derived DNA adducts is shown in Figure 1, frames A and B, respectively. The spectra were obtained at delay times of 0, 40, and 80 ns. Frame A shows that the position of the (0,0) band of the BPDE-10-N7Ade adduct is independent of the delay time of the observation window. In contrast, the origin band of the BP-6-N7Ade adduct strongly depends on the delay time, as shown in Frame B. A non-linear shift of the time resolved (0,0)-emission band was observed (data not shown). We attribute the observed behavior to the fact that the inhomogeneously broadened absorption band is characterized by different effective solute dipoles for which non-linear solvation effects

assume significance. Such a blue-shift and narrowing of the (0,0) band, to the best of our knowledge, has not been previously reported.

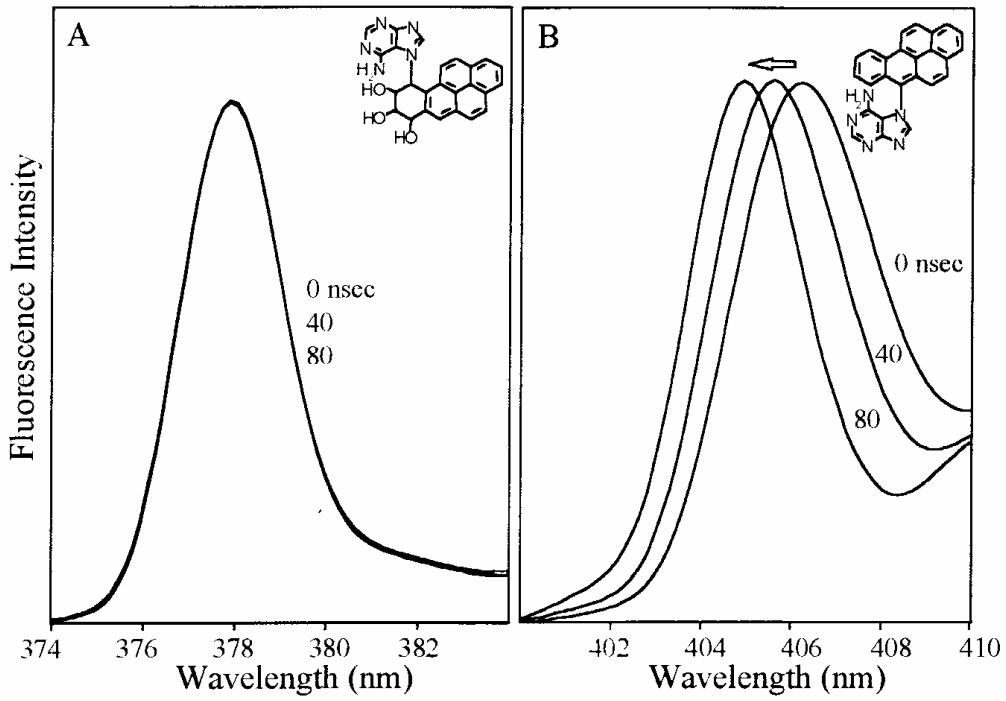


FIGURE 1 Comparison of the normalized fluorescence origin bands of the two BP-derived DNA adducts: Frame A) monooxygenation BPDE-10-N7Ade adduct; Frame B) one-electron oxidation BP-6-N7Ade adduct. T=77 K, λ_{ex} = 308 nm, gate width = 10 nsec.

To eliminate the possibility of sample contamination, capillary electrophoresis (CE) was used to confirm the purity of the BP-6-N7Ade adduct. A fluorescence electropherogram is shown in Figure 2. On-line detection using the CE-FLNS system revealed a single peak in the electropherogram and typical fluorescence spectra of the BP-6-N7Ade adduct (spectra not shown), establishing the purity of the analyte in question.

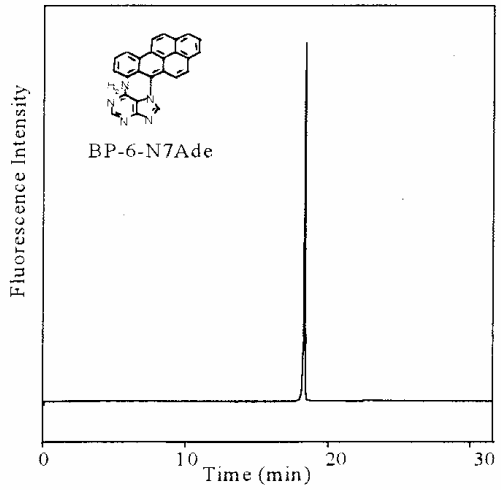


FIGURE 2 Room-temperature fluorescence electropherogram of the BP-6-N7Ade adduct, using a CE buffer consisting of 20 mM DOSS (sodium dioctyl sulfosuccinate) and 8 mM sodium tetraborate in acetonitrile-water (30:70, v/v). Voltage: 20 kV; current: ~20 μA; excitation: 351 nm, Ar ion laser (65 mW); capillary, 95 cm×75μm I.D; effective length 70 cm.

Spectra shown in Figure 1 suggest that the analysis of BP-6-N7Ade adduct standards and *in vivo* obtained samples, acquired for the same laser excitation frequency but different delay times and/or gate widths, should exhibit different FLN spectra. This is demonstrated in Figure 3, where spectra a-d are obtained with 10 ns gate width for 0, 20, 40, and 60 ns delay times, respectively. The narrowing of the FLN spectra, in agreement with Figure 1, indicates that at longer delay times the fluorescence originates from molecules absorbing at the high-energy wing of the inhomogeneously absorption band. The latter is in agreement with the observed distribution of fluorescence lifetimes (data not shown). Thus, we conclude that due to frequency-dependent fluorescence lifetimes, the high-resolution FLN spectra of standards

and *in vivo* obtained samples, at least for this class of molecules, have to be generated at identical conditions. This finding is very important since sometimes the *in vivo* samples are measured at longer delay times to avoid scattered laser light, which could lead to problems of equating the analyte of interest with a corresponding adduct standard.

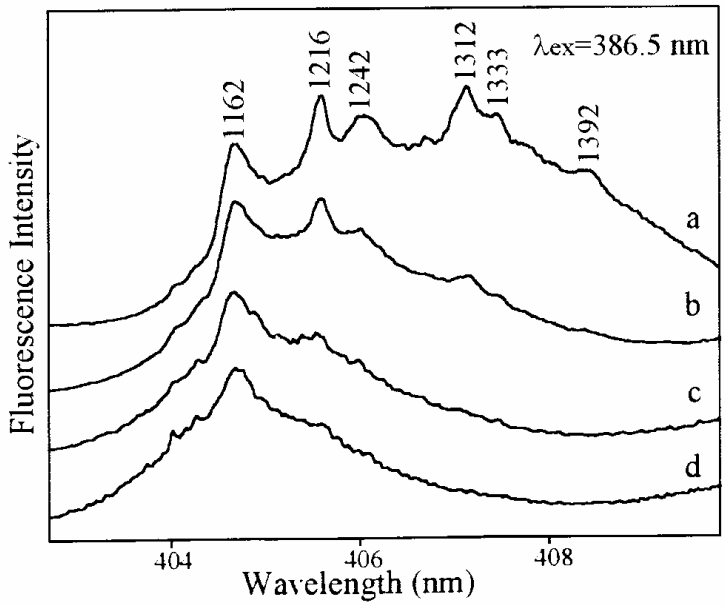
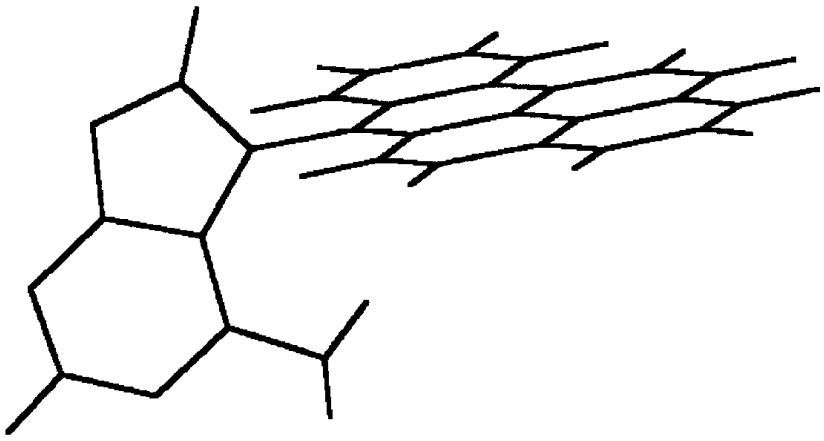


FIGURE 3 FLN spectra of the BP-6-N7Ade adduct in ethanol glass (T=4.2K, λ_{ex}=386.5 nm). Curve a: 0 ns; curve b: 20 ns; curve c: 40 ns; and curve d: 60 ns delay time.

Adduct Conformation

In order to shed more light on this unique behavior of the BP-6-N7Ade adduct, a molecular modeling study was initiated. A typical structure obtained from the molecular dynamic simulations (simulated rapid cooling to T= 0 K of various high-temperature conformations) and subsequent optimization is shown in Figure 4. The calculations indicate that a distribution of conformations having slightly different orientations of the adenine moiety is possible. The orientation of a

dipole moment may be correlated to a variation in the observed excited



state lifetime. A lifetime dependence on spectral shift was recently observed for dye molecules located at a polymer-air interface [7].

FIGURE 4 Optimized structure of the BP-6-N7Ade adduct.

Stokes Shift

Time-resolved fluorescence emission and fluorescence excitation spectra obtained at 0, 40, and 80 ns delay of the observation window are shown in Figure 5 (Frame A). The time-dependent Stokes shift, observed in ethanol glass at 77 K, is plotted in Frame B. These data clearly reveal a non-linear time-dependence of the reorganization energy. This behavior is consistent with a broad distribution of permanent dipole moment changes. To account for this behavior, two effects have to be considered in combination: a distribution of adduct conformers and a matrix-induced, frequency dependent, dipole moment change across the inhomogeneously broadened absorption band. Thus it is anticipated that the observed dipole moment changes depend on the configuration of the matrix fields around a particular

conformer of BP-6-N7Ade adduct. For the solvated adduct, equilibrated with water molecules, the effective dipole moment change is – 0.27 D. Using the latter value the calculated and measured Stokes shifts are in reasonable agreement, as will be shown in a forthcoming publication [8].

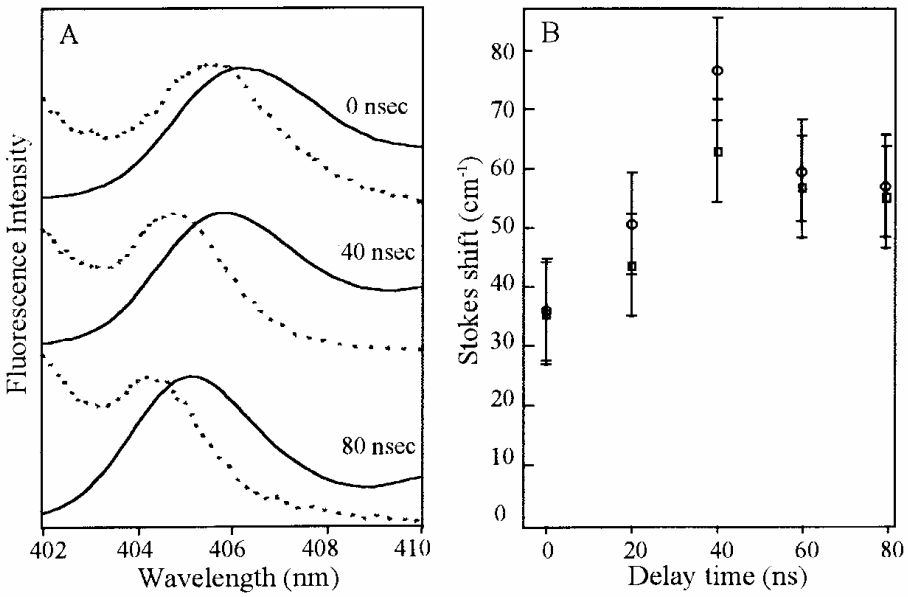


Figure 5 Time-dependent Stokes shift obtained for the BP-6-N7Ade adduct; T=77K, λ_{ex}=308 nm, gate width 10 ns. A) Time resolved fluorescence (solid lines) and fluorescence excitation (dotted) spectra. B) Stokes shift measured at different delay times of the observation window. Squares and circles were obtained with 5 and 10 ns gate width, respectively.

CONCLUSIONS

A time-dependent Stokes shift for the BP-6-N7Ade adduct, in ethanol glass at low temperature, has been revealed for the first time. This indicates that there must be a distribution of adduct conformers and a distribution of matrix-induced dipole moment changes. As a result,

high-resolution FLN spectra for this class of molecules have to be acquired under identical conditions in order to eliminate the effect of this time-dependent Stokes shift. We believe that future hole-burning and Stark spectroscopy experiments on the BP-6-N7Ade adduct will shed more light on this interesting issue.

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