Spectral and Conformational Analysis of Deoxyadenosine Adducts Derived from syn- and anti-Dibenzo[a,l]pyrene Diol Epoxides: Fluorescence Studies

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Low-temperature fluorescence spectroscopy and results of conformational studies with trans-syn-, cis-syn-, trans-anti-, and cis-anti-dibenzo[a,l]pyrene diol epoxide (DB[a,l]PDE)-derived deoxy-adenosine (dA) adducts are presented and compared with those previously obtained for the stereoisomeric DB[a,l]P tetrols [Jankowiak, R., et al. (1997) Chem. Res. Toxicol. 10, 677–686]. In contrast to DB[a,l]P tetrols, for which only trans isomers exhibited two conformers, all stereoisomeric dA adducts adopt two different conformations with either half-chair or half-boat structures for the cyclohexenyl ring, and an “open”- or “folded”-type configuration between dA and the DB[a,l]P moiety. The major conformations observed for trans-syn-, cis-syn-, and cis-anti-DB[a,l]PDE–14-N6dA could be assigned on the basis of the previous calculations for the DB[a,l]P tetrols. The major conformers of the trans-syn- and cis-syn-DB[a,l]PDE–14-N6-dA adducts exist in conformations I and II, with their fluorescence origin bands at ~382 and ~389 nm, respectively. In conformation I, the cyclohexenyl ring adopts a half-boat structure with dA in a pseudoaxial position (an open configuration), whereas the cyclohexenyl ring in conformation II adopts a half-chair structure with dA in pseudoequatorial position (a folded configuration). The major conformation of cis-anti-DB[a,l]PDE–14-N6dA, with its origin band at ~389 nm, was also assigned as a folded-type configuration with a half-chair structure in the cyclohexenyl ring. Molecular mechanics and dynamical simulations were performed for interpretation of the low-temperature fluorescence spectra and 1H NMR coupling constants observed for the trans-anti-DB[a,l]PDE–14-N6dA adduct. The major conformer of this adduct has a half-chair structure in the cyclohexenyl ring, but a deviation from planarity in the fjord region different from that of conformer II of cis-anti-DB[a,l]PDE–N6dA. This new structure is labeled as conformer II’. Its (0,0) fluorescence band is at 388.1 and 388.3 nm in ethanol and glycerol/water glasses, respectively, consistent with the folded-type configuration revealed by the calculations. The fluorescence line-narrowed spectra reveal that the trans-syn-, cis-syn-, trans-anti-, and cis-anti-DB[a,l]PDE–14-N6dA adducts can be distinguished. Thus, their spectra should prove useful for identification of DB[a,l]P–DNA adducts formed at low levels in biological samples.

Introduction

Dibenzo[a,l]pyrene (DB[a,l]P) is the most potent carcinogen among the polycyclic aromatic hydrocarbons (PAHs) (1, 2). It has been found in river sediment (3) and indoor (4) and outdoor (5) air samples, suggesting potential (eco)toxicological hazards. DB[a,l]P can be enzymatically activated by two main pathways: one-electron oxidation to yield radical cations (6–9) and monooxygenation to produce bay-region diolepoxides (10–15). Numerous DB[a,l]P–DNA adducts have been reported (6–16).

Low-temperature fluorescence spectroscopy has proven to be a valuable tool for DNA adduct characterization.

In particular, fluorescence line-narrowing spectroscopy (FLNS) (8, 17) has been used for definitive identification (6, 8–24) of adducts. Furthermore, the combination of FLNS and non-line-narrowing (NLN) fluorescence spectroscopy can provide adduct conformational information.

Abbreviations: CE, capillary electrophoresis; dAMP, deoxyadenosine monophosphate; DB[a,l]P, dibenz[a,l]pyrene; DB[a,l]PDE, dibenz[a,l]pyrene diol epoxide; DB[a,l]PDE–14-N6dG, dibenz[a,l]pyrene diol epoxide–N6-deoxyguanosine; syn-DB[a,l]PDE–14-N6dA, syn-dibenzo[a,l]pyrene diol epoxide–14-N6-deoxyadenosine; anti-DB[a,l]PDE–14-N6dA, anti-dibenzo[a,l]pyrene diol epoxide–14-N6-deoxyadenosine; DB[a,l]PDE–14-N7Gde, 14-(adenin-7-yl)-11,12,13-trihydroxy-11,12,13,14-tetrahydrodibenzo[a,l]pyrene; DB[a,l]PDE–14-N7Gua, 14-(guanin-7-yl)-11,12,13-trihydroxy-11,12,13,14-tetrahydrodibenzo[a,l]pyrene; DB[a,l]PDE–14-N7Gde, 11,12,13,14-tetrahydrodibenzo[a,l]pyrene; DE, dial epoxide; FLNS, fluorescence line-narrowing spectroscopy; g/w, glycerol/water; MD, molecular dynamics; Me2SO, dimethyl sulfoxide; MM, molecular mechanics; PAH, polycyclic aromatic hydrocarbon; Sc, state, electronic ground state; S1, state, lowest excited singlet state; ZPL, zero-phonon lines.
The insets show the conformation of the cyclohexenyl ring (half-folded-type conformation II (frame B), Ade is in a pseudo-shepherd's crook conformation II, and the aromatic system is possible. In the open-type adduct structure (frame A), the cyclohexenyl ring adopts a half-chair structure where no significant interaction between the adenine (Ade) and the aromatic system is possible. In the folded-type conformation II (frame B), Ade is in a pseudo-equatorial position and the cyclohexenyl ring adopts a half-chair structure. Similar conformations were observed for trans-syn-DB[a,l]P diol tetrols (26). The calculated and observed fluorescence origins bands established for various conformations of the trans-syn-, cis-syn-, trans-anti-, and cis-anti-DB[a,l]P tetrols, as well as the calculated dihedral angles and the estimated 1H NMR coupling constants for the proton pairs of the cyclohexenyl ring, are summarized in Table 1. For stereoisomeric DB[a,l]P tetrols, the agreement between the measured and theoretically estimated NMR coupling constants suggests that the results shown in Table 1 may be useful for interpretation of the spectroscopic data obtained for DB[a,l]PDE-dA adducts.

In this work, DB[a,l]PDE-dA adducts, for which eight stereochemical configurations are shown in Figure 2, were studied by low-temperature fluorescence spectroscopy and molecular modeling. The NLN and FLN spectra of trans-anti, cis-anti, trans-cis, and cis-syn-DB[a,l]PDE-14-NdA adducts presented below provide the necessary spectral information for investigating the nature of DB[a,l]PDE-NA adducts formed at low levels in in vitro and in vivo studies. The structural characterization of these adducts by NMR, circular dichroism, and fast atom bombardment mass spectrometry is presented elsewhere.

**Materials and Methods**

Caution: anti- and syn-DB[a,l]P diol epoxides are extremely hazardous chemicals and should be handled carefully in accordance with NIH guidelines.

**Sample Preparation.** The DB[a,l]PDE-derived adducts trans-anti-, cis-anti-, trans-cis-, and cis-syn-DB[a,l]PDE-14-NdA were synthesized by the reaction of anti- and syn-DB[a,l]PDE with dA. The (±)-anti-DB[a,l]PDE was reacted with dA in dimethylformamide at 100 °C for 30 min to give four anti-DB[a,l]PDE-14-NdA adducts. The (±)-syn-DB[a,l]PDE was reacted with dA under the same conditions to yield the four syn-DB[a,l]PDE-14-NdA adducts. For details on the synthesis and structural characterization, see the paper by K.-M. Li et al.

**Adduct Purity.** The purity of dA standards separated by HPLC was checked by capillary electrophoresis (CE), which possesses higher separation power (i.e., higher efficiency) than HPLC. A mixture of 85% A (40 mM dioctyl sulfosuccinate and 8 mM sodium borate in 30% (by volume) acetonitrile/70% water (pH 9)) and 15% B (50 mM Brij 5) was used as the CE buffer. These conditions allowed for separation of all eight diastereomers. Figure 3 shows room-temperature absorbance electropherograms of HPLC-separated (−)-trans-anti- (a), (−)-cis-anti- (b), (−)-trans-cis- (c), and (+)-cis-syn-DB[a,l]PDE-14-NdA (d). The results establish that the purity levels are very high, and thus, one can be confident that the library of NLN and FLN spectra that was obtained is reliable.

**Low-Temperature Fluorescence Spectroscopy.** NLN fluorescence spectra at 77 K and FLN spectra (S1 → S0 excitation) at 4.2 K were obtained using a Lambda Physik FL-2002 dye laser pumped by a Lambda Physik Lextra 100 XeCl excimer laser as the excitation source. For FLN spectroscopy, several excitation wavelengths were used, each of which reveals a portion of the S1 excited state vibrational frequencies of the...
TABLE 1. Calculated and Observed (0,0) Transition Energies, Dihedral Angles $\alpha$ and $\beta$ with the Estimated Coupling Constants for the (i,j) Proton Pairs, and Structure Assignments for Various Conformations of syn- and anti-DB[a, l]P Tetrols$^a$

<table>
<thead>
<tr>
<th>DB[a, l]P tetrol conformation</th>
<th>$\lambda_{calc}^a$ (0,0) (nm)</th>
<th>$\lambda_{obs}^c$ (0,0) (nm)</th>
<th>$\alpha^d$ (deg)</th>
<th>$\beta^d$ (deg)</th>
<th>structure assignment$^f$</th>
<th>coupling constants$^g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-syn-</td>
<td>381.4</td>
<td>382.2 (EtOH), 382.7 (g/w)</td>
<td>25</td>
<td>8</td>
<td>half-boat, pseudoaxial</td>
<td>L/L/S/S</td>
</tr>
<tr>
<td>cis-syn-</td>
<td>384.0</td>
<td>387.0 (g/w)</td>
<td>26</td>
<td>60</td>
<td>half-chair, pseudoequatorial</td>
<td>L/L/L/S</td>
</tr>
<tr>
<td>trans-anti-</td>
<td>384.5</td>
<td>385.2 (EtOH), 385.5 (g/w)</td>
<td>26</td>
<td>60</td>
<td>half-chair, pseudoequatorial</td>
<td>S/S/S/S</td>
</tr>
<tr>
<td>cis-anti-</td>
<td>384.4</td>
<td>385.4 (g/w)</td>
<td>27</td>
<td>59</td>
<td>half-chair, pseudoequatorial</td>
<td>S/S/S/S</td>
</tr>
<tr>
<td>trans-a</td>
<td>382.0</td>
<td>382.2 (EtOH), 383.2 (g/w)</td>
<td>24</td>
<td>25</td>
<td>flattened, pseudoaxial</td>
<td>M/M/S/M/S</td>
</tr>
<tr>
<td>cis-a</td>
<td>384.3</td>
<td>-</td>
<td>24</td>
<td>63</td>
<td>half-chair, pseudoequatorial</td>
<td>L/S/S/S</td>
</tr>
</tbody>
</table>

$^a$ Data from ref 26. $^b$ These conformations were the most consistent with the room-temperature $^1$H NMR data. $^c$ Spectroscopically observed (0,0) transition energies in ethanol (EtOH) or glycerol/water (g/w) at 77 K. $^d\alpha$ describes the deviation from planarity in the fjord region, and $\beta$ describes the conformation of the cyclohexenyl ring; see Figure 1. $^e$ The observed fluorescence (0,0) bands may correspond to either the $\gamma$ or the $\alpha$' conformation. $^f$ Conformation of the cyclohexenyl ring and orientation of the hydroxyl group at the C-14 position. $^g$ Estimated coupling constants for the (11,12), (12,13), and (13,14) proton pairs. L is large, vL very large, M medium, and S small; for details, see ref 26.

Figure 2. Molecular structures of the eight DB[a, l]PDE - 14-N$^d$A adducts that were investigated: (A) (±)-trans-syn-DB[a, l]PDE - 14-N$^d$A, (B) (±)-cis-syn-DB[a, l]PDE - 14-N$^d$A, (C) (±)-trans-anti-DB[a, l]PDE - 14-N$^d$A, and (D) (±)-cis-anti-DB[a, l] PDE - 14-N$^d$A. The dihedral angles $\alpha$, $\beta$, and $\gamma$ will be used to describe the deviation from planarity in the fjord region, the conformation of the cyclohexenyl ring, and the orientation of the da moiety, respectively. dR represents deoxyribose.

Analyte (only selected spectra are presented). NLN spectra were obtained using nonselective excitation at 308 nm from the excimer laser. Samples were cooled in a glass cryostat with quartz optical windows. Fluorescence was dispersed by a Princeton Instruments FG-100 pulse generator was employed; different detector delay times (0–60 ns) with a gate width of 200 ns were used. The resolution for FLN and NLN spectra was 0.05 and 0.8 nm, respectively. Two solvent matrices with different polarities were used: ethanol and a mixture of glycerol/water (50/50 v/v). Ethanol was spectrophotometric grade from Aldrich. Ultrapure grade glycerol was purchased from Spectrum Chemical (Gardena, CA). Samples (ca. 20 µL) were transferred to quartz tubes (2 mm i.d.) and the tubes sealed with a rubber septum. Adduct concentrations were in the $10^{-6}$ M range.

Molecular Mechanics. Conformational analyses were carried out utilizing methods of molecular mechanics (MM), wherein energy calculations were performed with HyperChem's molecular modeling program (Release 5.1 for Windows Hypercube Inc.). HyperChem's force field (MM +/+) developed for organic molecules (27, 28) was employed utilizing default parameters. As starting structures for the trans-anti-DB[a, l]PDE - 14-N$^d$A adduct, different model-built configurations in which the saturated ring was in either a half-chair or half-boat conformation were used. The Polak–Ribiere algorithm (in vacuo) was used for molecular mechanics optimization; the structures were
We have previously reported for benzo[a]pyrene diol epoxide-derived adducts that the (+)- and (−)-trans or (+) and (−)-cis nucleoside enantiomers cannot be distinguished from one another by fluorescence methods (23). True (+)- and (−)-enantiomers cannot be distinguished since they are related by reflection (mirror) symmetry. Apparently, in the case of these DB[a,l]PDE–dA nucleotide adducts, the influence of the sugar moiety on the fluorescence characteristics is negligible. It is worthy to note that, due to the deoxyribose ring, these (+)- and (−)-dA adducts are not true enantiomers, but rather diastereomers. In what follows, detailed characterization of dA adducts obtained with the optically pure (−)-anti-DB[a,l]PDE and (−)-syn-DB[a,l]PDE enantiomers will be discussed for both trans- and cis-opening dA adducts. Identical data for (−)-anti-DB[a,l]PDE and (−)-syn-DB[a,l]PDE enantiomers were also obtained (not shown).

NLN spectra of trans-, cis-, trans-anti-, and cis-anti-DB[a,l]PDE–14-N-dA adducts are shown in frames A–D of Figure 4, respectively. The major differences between the spectra in Figure 4 are revealed by the spectral position of the (0,0) bands, the intensity distribution of the vibronic bands, and the relative distribution of adduct conformations. The origin bands labeled as (0,0) or (0,0)v indicate that they belong to different molecular conformations, vide infra. As was the case for trans-anti- and trans-syn-DB[a,l]P tetrol isomers (see Table 1), the NLN spectra of the dA adducts are also solvent-dependent. As a result, each of these adducts may exist in a conformation having its origin band at 382–385 nm (labeled as conformation I) and/or in a conformation having its origin band at ~388–390 nm (denoted as conformation II) with the ratio of I/II being solvent-dependent. Additionally, variations in the vibronic intensity distribution and the S0 vibrational frequencies (Figure 4) are not surprising given that the parent fluorophore B[e]P has C2v symmetry, and the out-of-plane deformation, as well as the conformation of the cyclohexenyl ring, should depend on adduct stereochemistry as was observed in the case of the stereoisomeric DB[a,l]P tetrols (26).

**Results and Discussion**

DB[a,l]PDE-derived dA adducts, which were isolated from reaction mixtures in which both the racemic mixture and the optically pure syn- and anti-DB[a,l]PDE were reacted with Ade, were studied. The NLN and FLN spectra obtained for adducts formed with the racemic mixtures of the respective diol epoxides gave four pairs of identical spectra (not shown) with the pairs corresponding to (+)- and (−)-enantiomers of trans-syn-, cis-syn-, trans-anti-, and cis-anti-DB[a,l]PDE–14-N-dA adducts. These identical fluorescence spectra for (+) and (−)-enantiomers, for a given adduct, were expected since we have previously reported for benzo[a]pyrene diol epoxide-derived adducts that the (+)- and (−)-trans or (+)- and (−)-cis nucleoside enantiomers cannot be distinguished from one another by fluorescence methods (23). True (+)- and (−)-enantiomers cannot be distinguished since they are related by reflection (mirror) symmetry. Apparently, in the case of these DB[a,l]PDE–dA nucleotide adducts, the influence of the sugar moiety on the fluorescence characteristics is negligible. It is worthy to note that, due to the deoxyribose ring, these (+)- and (−)-dA adducts are not true enantiomers, but rather diastereomers. In what follows, detailed characterization of dA adducts obtained with the optically pure (−)-anti-DB[a,l]PDE and (−)-syn-DB[a,l]PDE enantiomers will be discussed for both trans- and cis-opening dA adducts. Identical data for (−)-anti-DB[a,l]PDE and (−)-syn-DB[a,l]PDE enantiomers were also obtained (not shown).

N LN spectra of trans-, cis-, trans-anti-, and cis-anti-DB[a,l]PDE–14-N-dA adducts are shown in frames A–D of Figure 4, respectively. The major differences between the spectra in Figure 4 are revealed by the spectral position of the (0,0) bands, the intensity distribution of the vibronic bands, and the relative distribution of adduct conformations. The origin bands labeled as (0,0) or (0,0)v indicate that they belong to different molecular conformations, vide infra. As was the case for trans-anti- and trans-syn-DB[a,l]P tetrol isomers (see Table 1), the NLN spectra of the dA adducts are also solvent-dependent. As a result, each of these adducts may exist in a conformation having its origin band at 382–385 nm (labeled as conformation I) and/or in a conformation having its origin band at ~388–390 nm (denoted as conformation II) with the ratio of I/II being solvent-dependent. Additionally, variations in the vibronic intensity distribution and the S0 vibrational frequencies (Figure 4) are not surprising given that the parent fluorophore B[e]P has C2v symmetry, and the out-of-plane deformation, as well as the conformation of the cyclohexenyl ring, should depend on adduct stereochemistry as was observed in the case of the stereoisomeric DB[a,l]P tetrols (26).
the red-shifted origin band. The NMR coupling constants obtained for the trans-syn-DB[a,]IPDE—dA adduct ([J]_{11,12} 
~ 7 Hz, [J]_{12,13} = 9.0 Hz, and [J]_{13,14} = 8.0 Hz) are consistent with the above half-chair assignment predicted by dynamical simulation for trans-syn-DB[a,]IP tetrol, for which the estimated coupling constants of the (11,12), (12,13), and (13,14) proton pairs were large, very large, and very large, respectively. We conclude, therefore, that trans-syn-DB[a,]IPDE—dA, as observed in room-temperature NMR spectra (in Me$_2$SO solvent), exists in folded-type conformation II.

The existence of the above-discussed conformers is confirmed by FLN spectra. Multiplet origin structures for the trans-syn-DB[a,]IPDE—14-N$_6$dA adduct in ethanol (spectra a and c) and in glycerol/water glass (spectra b, d, f, and h, respectively). T = 77 K. T$_{ex} = 308$ nm. Delay time = 20 ns. Gate width = 200 ns. The numbers correspond to the ground state (S0) vibrational frequencies.

**Table 2. Fluorescence Characterization and Conformational Analysis of syn- and anti-DB[a,]IPDE—14-N$_6$dA Adducts**

<table>
<thead>
<tr>
<th>stereoisomeric adduct</th>
<th>ethanol</th>
<th>glycerol/water</th>
<th>assignment</th>
</tr>
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<tbody>
<tr>
<td>(±)-trans-syn-dA</td>
<td>382.0</td>
<td>I</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>382.0 I</td>
</tr>
<tr>
<td>(±)-cis-syn-dA</td>
<td>383.3</td>
<td>I</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>383.3 I</td>
</tr>
<tr>
<td>(±)-trans-anti-dA</td>
<td>383.0</td>
<td>I</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>383.0 I</td>
</tr>
<tr>
<td>(±)-cis-anti-dA</td>
<td>385.0</td>
<td>I</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>385.0 I</td>
</tr>
</tbody>
</table>

a The bold Roman numerals denote the major conformations observed by low-temperature fluorescence. b Conformation of the cyclohexenyl (nonaromatic benzylic) ring and the orientation for the dA moiety; $\alpha$, $\beta$, and $\gamma$ are defined in Figure 1. c Minor conformation at the nucleoside level, but major conformation in single-stranded DNA (16) and in CE buffer solution. d In double-stranded DNA, this adduct adopts an intercalated conformation II (16) (see the text for details). e Very weak, so cis-anti-DB[a,]IPDE—14-N$_6$dA in glycerol/water glass exists mostly in conformer II (see Figures 4D and 10).
excited state mode frequencies at 766, 794, 863, and 924 cm\(^{-1}\) are typical for conformer I, while modes at 785, 862, 932, and 961 cm\(^{-1}\) are observed for conformer II. These results suggest that the molecular conformations of conformer I and II are different. The same conclusion was reached on the basis of results presented in Figure 4A and calculations performed for trans-syn-DB[a,l]P tetrol (26), which indicate that the major conformation (conformer I) of the trans-syn-dA adduct has the cyclohexenyl ring in a half-boat structure. However, at room temperature the major conformation observed, as shown by \(^1\)H NMR spectroscopy,\(^3\) is conformation II with a half-chair structure for the cyclohexenyl ring, and a folded-type structure with dA in a pseudoequatorial position. The latter is consistent with the large experimentally observed red shift (~470 cm\(^{-1}\)) of the (0,0) band.

Frame B of Figure 4 shows NLN spectra of the (+)-cis-syn-DB[a,l]PDE–dA adduct in ethanol (curve c) and glycerol/water (curve d), respectively. In ethanol, the (0,0) band is located at 383.6 nm, while in glycerol/water, it is at 384 nm. The small spectral shift of 0.4 nm is due to the solvent effect. However, a small contribution from the red-shifted conformer II, with its origin band at ~388 nm, is also revealed in both solvents. Unlike conformer I of trans-syn-DB[a,l]PDE–dA in ethanol, both conformations of the cis isomer have a weak (0,0) band and very intense Herzberg–Teller origin band at ~760 cm\(^{-1}\). The significant intensity of this band is attributed to electronic vibrational coupling between the S\(_1\) and higher-energy dipole-allowed states, and is a consequence of the S\(_1\) − S\(_0\) absorption transition being only weakly allowed (31).

For cis-syn-DB[a,l]P tetrol, only conformer I (26) was observed experimentally. However, modeling studies suggested that in vacuo cis-syn-DB[a,l]P tetrol may exist in two different half-chair conformations (26). Thus, on the basis of ref 26 and Table 1, the minor conformation of cis-syn-dA (conformer II having its (0,0) band at 388 nm) is tentatively assigned as a half-chair structure for the cyclohexenyl ring (negative \(\beta\) value) with dA in a folded-type geometry. The main conformation [conformer I with its (0,0) band at 384 nm] is assigned as a different half-chair (with a positive \(\beta\) value) and dA in an open-type configuration. The latter assignment is in good agreement with the \(^1\)H NMR coupling constants for the proton pairs of the cyclohexenyl ring with \(J_{11,12}, J_{12,13}, J_{13,14}\) being ~7 Hz (large), 7.5 Hz (large), and ~3 Hz (small), respectively.\(^2\)

In Figure 6, FLN spectra for the cis-syn-dA adduct, obtained with excitation at 378.0 nm, are presented. Spectra a and b were obtained in ethanol and glycerol/water glasses, respectively. Again, comparison of these spectra shows no differences in vibrational frequencies for conformer I [within the (0,0) spectral range], proving that this conformation is the same in both glasses. The higher relative intensities of the 270 and 428 cm\(^{-1}\) modes in glycerol/water glass are, as in the case of the trans-syn-isomer, due to larger inhomogeneous broadening observed in glycerol/water glass. However, in glycerol/water, a relatively large contribution from adduct conformation II, having its origin band red-shifted to ~389 nm, is also observed. This is in agreement with data presented in Figure 4B (spectrum d).
anti-DB[a,l]PDE–dA Adducts. Frames C and D of Figure 4 show NLN fluorescence spectra for trans-anti- and cis-anti-DB[a,l]PDE–dA adducts. Spectra e and g and f and h were obtained in ethanol and glycerol/water glasses, respectively. In contrast to the syn-DB[a,l]PDE-derivadducts, the red-shifted conformation is clearly observed for both trans-anti- and cis-anti-dA adducts. Figure 4C shows that the major conformer for trans-anti-DB[a,l]PDE–dA, in both solvents, has its origin band at \( \sim 389 \text{ nm} \). Also, in this case, the Herzberg–Teller origin band at \( \sim 770 \text{ cm}^{-1} \) is the most intense. The coupling constants previously calculated for conformer I (with a large \( J_{11,12} \), a small \( J_{12,13} \), and a small \( J_{13,14} \)) and conformer II (with a small \( J_{11,12} \), a small \( J_{12,13} \), and a large \( J_{13,14} \)) of trans-anti-DB[a,l]PDE tetrol (26) cannot explain the proton NMR coupling constants measured for the (+)-trans-anti-dA adduct, which are as follows: \( J_{11,12} = 8.0 \text{ Hz (large)}, J_{12,13} = 6.0 \text{ Hz (medium)}, \) and \( J_{13,14} = 5.0 \text{ Hz (medium)} \). The \( J_{12,13} \) and \( J_{13,14} \) coupling constants for the (–)trans-anti-dA adduct were not well resolved in Me2SO, and thus cannot be directly compared with those of (+)-trans-anti-dA. Nonetheless, we emphasize that circular dichroism,3 NLN, and FLN spectra for (+)- and (–)-diastereomers were identical (data not shown), proving that the (+)- and (–)-trans-anti-DB[a,l]PDE–14-N7Ade adducts do exist in the same conformation.

To interpret the above data for trans-anti-DB[a,l]-PDE–14-N7Ade adducts, a theoretical investigation was initiated utilizing MM and MD simulations. The minimum energy of the major conformation observed in MD simulations was 33.4 kcal/mol. Its structure is shown in Figure 7, which indicates that this adduct exists in a folded-type conformation. The dihedral angles \( \alpha \) and \( \beta \) are both positive, with values of 30.9 and 59°, respectively. Angle \( \alpha \), defined as \( C_{14a}–C_{14b}–C_{14c}–C_{1} \), describes a propeller-like distortion of the DB[a,l]P moiety that relieves the strain of the sterically hindered fjord region of the DB-[a,l]P residue by minimizing the steric repulsion between the H1 and H14 protons. Similar values of \( \alpha \) were observed for trans-syn-DB[a,l]PDE–14-N7Ade (24) and benzo[...]naphthrene diol epoxide adducts (32). On the other hand, the \( \alpha \) and \( \beta \) values for trans-anti-DB[a,l]P tetrol, as shown in Table 1, are –24 and 60°, and 27 and –63° for conformers I, and II, respectively (26). The conformation of the trans-anti-DB[a,l]-PDE–dA adduct, due to specific steric hindrance created by the fjord region of DB[a,l]P, exists in a conformation with positive \( \alpha \) and \( \beta \) values, and is termed conformer II′ (Table 2).

Another unique conformation of trans-anti-dA (with a similar local energy minimum of \( \sim 34 \text{ kcal/mol} \)) was also observed in the simulations. In this conformation, the \( \alpha \) and \( \beta \) values are 28 and –63.7° with the DA moiety in a pseudodaxial position, respectively, thus leading to the open-type structure with a large \( \gamma \) value of 158.4°. Consequently, no significant interaction between DA and the aromatic system is possible. We associate this structure with the experimentally observed minor conformer (I′) having its origin band at 383.0 nm. Although this conformer is hardly observed in ethanol (see Figure 4C), it is preferentially formed in a micellar CE buffer matrix. The FLN spectra obtained for the trans-anti-dA isomer are shown in Figure 8; frames A and B were obtained for two different excitation wavelengths, 374.0 and 378.0 nm, respectively. Comparison of spectra A and B (with spectra c and d (glycerol/water) indicates that the major, red-shifted, conformer II′ is the same in both glasses. The weak modes at 422, 549, and 627 cm\(^{-1} \) (spectrum a) correspond to the minor conformer I′ with its origin band at 383.0 nm.

The NLN spectra for the cis-anti-DB[a,l]PDE–dA adduct (Figure 4D), in contrast to those for cis-anti-DB-
[a,l]P tetrol (26), imply that two conformers may exist in ethanol, while only one conformer is observed in the glycerol/water glass. The origin bands of these conformers are at 385.4 and 389.8 nm, respectively. Room-temperature NMR data obtained in Me2SO revealed the presence of only one conformation with a large J, a small J, and an undetermined J. These coupling constants, based on the calculations for cis-anti-DB[a,l]P tetrol (26) and preliminary data for the cis-anti-DB[a,l]-PDE–dA adduct (data not shown), are consistent with conformation II in which the cyclohexenyl ring adopts a half-chair structure with a positive value of the dihedral angle β. The blue-shifted conformation with dA in an open-type configuration.

Time-resolved spectroscopy revealed the cis-anti-dA adducts in conformation I possess a different fluorescence lifetime compared to adducts in conformation II, so the fraction of adducts in conformation I can be resolved. The spectrum obtained as the difference between two delay times (60 and 20 ns) of the observation window is shown in Figure 9. This temporal difference spectrum reveals that the adducts in conformation I have a longer fluorescence lifetime. As a result, only the origin band at 385.0 nm and its corresponding vibronic progression are exposed. This is in contrast to spectrum g of Figure 4D, where both conformers and their vibronic modes are observed.

The FLN spectra in Figure 10 for the cis-anti-isomer also suggest the presence of two unique conformations, consistent with the data depicted in Figure 4D. Comparison of the vibrational frequencies (~850–1100 cm⁻¹) in spectra b of Figures 8A and 10 showed that the redshifted conformers of the trans-anti- and cis-anti-dA adducts have different vibrational frequencies (e.g., 926 and 966 cm⁻¹ vs 929 and 960 cm⁻¹, respectively). This supports our earlier assignment that conformation II' of the trans-anti- and conformer II of the cis-anti-DB[a,l]PDE–N²dA isomers are clearly not the same.

Comparison of trans-syn- and cis-syn- versus trans-anti- and cis-anti-DB[a,l]PDE–N²dA adducts. Figure 11 shows four FLN spectra (in glycerol/water glass) obtained under identical conditions for trans-syn-(a), cis-syn- (b), trans-anti- (c), and cis-anti-DB[a,l]PDE–N²dA (d) diasteromers, respectively. These data show that the syn-type adducts (frame A), with ZPL at 387,
distinguished by FLN spectroscopy.

anti and trans conformers I (major) and II (minor).

These results show that observed variations are considered to be significant.

It was shown that both open-type (I and I′) and folded-type configurations (II and II′) could be formed. Comparison of the fluorescence origin bands (Table 2) reveals that in a glycerol/water glass, T = 4.2 K. λex = 376 nm. ZPL are labeled with their excited state vibrational frequencies, in cm⁻¹.

407, 436, 463, and 498 cm⁻¹, are indicative of trans-syn-dA adducts, while strong lines at 428, 472, 506, and 555 cm⁻¹ are characteristic of the cis-syn-dA isomers. A different pattern of zero-phonon lines is observed in frame B for trans- and cis-anti-dA adducts, spectra c and d, respectively. Here the characteristic modes are 740, 770, 800, and 838 cm⁻¹ for trans- and 745, 796, 854, 929, and 960 cm⁻¹ for cis-anti-dA. Differences were also observed in other frequency regions (data not shown).

With an experimental uncertainty of ±3 cm⁻¹, the observed variations are considered to be significant. These results show that trans- and cis-isomers of the syn- and anti-DB[a,1]PDE-derived dA adducts are readily distinguished by FLN spectroscopy.

Conclusions

We have demonstrated, using low-temperature fluorescence spectroscopy and computational chemistry, that not only the major but also the minor DB[a,1]PDE-derived dA adduct conformations can be characterized. Conformational data, including the results from molecular modeling and solvent-dependent studies performed for the diastereomeric DB[a,1]PDE–N⁴dA adducts, provided insight into possible conformations of the cyclohexenyl ring and the orientation of the deoxyadenosine moiety. It was shown that both open-type (I and I′) and folded-type structures (II and II′) could be formed. Comparison of the fluorescence origin bands (Table 2) reveals that in a glycerol/water glass trans-syn- and cis-syn-dA isomers adopt mostly conformation I, while trans-anti- and cis-anti-dA isomers exist mostly in conformations II′ and II, respectively. The major low-temperature conformations of cis-syn- (I), trans-anti- (I′), and cis-anti-dA (II) observed by fluorescence are compatible with the ²H NMR data. However, for trans-syn-DB[a,1]PDE–N⁴dA, the ²H NMR data show only conformer II, while the low-temperature fluorescence results show a mixture of conformers I (major) and II (minor).

The major conformers observed for trans-anti-dA (II′) and cis-anti-dA (II) are assigned as folded-type configurations with the same structure for the cyclohexenyl ring (positive β), opposite signs of α, and dA in a pseudoequatorial position partially stacked over the distal ring (see Table 2). The stacking leads to the experimentally observed red shift of the fluorescence origin bands. In contrast, the minor conformations of the above two isomers (I′ and I) are characterized by a different half-chair (negative β) with the dA moiety in a pseudoaxial position. Both minor conformers appear to exhibit similar deviation from planarity in the fjord region (positive α), as shown in Table 2.

In contrast to the trans-anti- and cis-anti-DB[a,1]PDE–N⁴dA adducts, where the major conformations have a half-chair structure in the cyclohexenyl ring, conformers I and II of trans-syn-dA feature half-boat and half-chair structures, respectively, with different orientations of the dA moiety.

The cis-syn-dA adduct with the half-chair cyclohexenyl ring in conformers I (open) and II (folded), and α and β being positive and negative and positive and positive, respectively, is in agreement with the cis-syn-DB[a,1]P tetrol calculations (26). The different vibrational patterns in the FLN spectra can provide a means of distinguishing the trans- and cis-isomers for both syn- and anti-DB[a,1]PDE–14-N⁴dA adducts. It is anticipated that these high-resolution FLN spectra will prove useful for future identifications of DB[a,1]PDE–DNA adducts (at the dAMP level) formed in biological systems.

These fluorescence results establish that anti-DB[a,1]PDE-derived dA adducts, the major adducts formed in mouse skin and calf thymus DNA (16), preferentially adopt conformation II (or II′) with origin bands at ~386–390 nm. The large red shift of the (0,0) band is in agreement with modeling studies, which indicate that these conformers exist in a folded-type geometry with significant π–π interactions. This suggests that molecular conformations of dA adducts may be important for understanding the preference of the bound metabolite toward external, base-stacked, and intercalated conformations, which were recently observed in DNA (16, 25).

Specifically, it was shown that in mouse skin the majority of anti-DB[a,1]PDE-derived DNA adducts adopt intercalated conformations, which in turn may influence their recognition by repair enzymes (25, 33). The analysis of mouse skin DNA exposed to DB[a,1]P, which showed that external adducts are repaired more efficiently than intercalated adducts (16), accentuates the importance of adduct conformation. The conformational data presented in this paper suggest the majority of anti-DB[a,1]PDE–14-N⁴dA adducts, due to a folded-type configuration, may be easily accommodated by the double helix of DNA. Moreover, the fact that the anti-dA adducts assume a folded-type configuration is consistent with the significant red shift of the fluorescence origin band of intercalated anti-DB[a,1]PDE–dA adducts observed for intact DNA (16). Therefore, we conclude that the large shifts (to ~398 nm) of the fluorescence origin bands of DNA adducts observed in ref 16 are caused by type II (or II′) conformers, which allow intercalation and, as a result, strong π–π interactions between the adduct and the DNA bases. In addition, it was shown here that the cis-anti-DB[a,1]PDE–14-N⁴dA adduct forms an open-type structure (I), implying that this adduct adopts an external conformation with intact DNA, consistent with our

Figure 11. FLN spectra of trans-syn- (curve a), cis-syn- (curve b), trans-anti- (curve c), and cis-anti-DB[a,1]PDE–N⁴dA adducts in a glycerol/water glass. T = 4.2 K. λex = 376 nm. ZPL are labeled with their excited state vibrational frequencies, in cm⁻¹.


earlier findings (16). Hence, it is entirely possible that the diverse structural conformations formed by DB[a,l]PDE-derived DNA adducts are responsible for the high carcinogenic potency of DB[a,l]P.

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