Conformational studies of depurinating DNA adducts from syn-dibenzo[α,β]pyrene diolepoxide

F. Ariese, G.J. Small and R. Jankowiak

Ames Laboratory-USDOE and Department of Chemistry, Iowa State University, Ames, IA 50011, USA

1To whom correspondence should be addressed

One of the major DNA adducts from the extremely potent aromatic carcinogen dibenzo[α,β]pyrene (DB[α,β]P) is the depurinating adduct syn-DB[α,β]P diolepoxide-14-N7 Ade. Low-temperature fluorescence spectra of this adduct (and related derivatives bound to N3-adenine and N7-guanine) showed two distinct (0,0) origin bands with different excited-state vibrational frequencies, as measured by means of fluorescence line narrowing spectroscopy. The relative intensity of the two origin bands was solvent dependent. The hypothesis that this phenomenon could be due to a conformational equilibrium was tested using molecular mechanics, dynamical simulations and semi-empirical quantum-mechanical calculations. The hydrolyzed metabolite DB[α,β]P tetraol was also studied for comparison. It was found that the syn-DB[α,β]P diolepoxide-14-N7 Ade adduct is formed via trans addition to the epoxide. Exploration of the conformational space indeed produced two potential energy minima; both corresponding to structures in which the aromatic ring system is severely distorted. In conformation I the proximity of the distal ring forces the adenine base into a pseudo-axial position and the cyclohexenyl ring adopts a half-chair structure. In conformation II the distal ring is bent in the opposite direction, allowing the cyclohexenyl ring to adopt a half-chair structure with the base in a pseudo-equatorial position, partially stacked over the distal ring. The difference in (0,0) transition energy calculated for the two conformers agrees very well with the spectroscopic data, and the relative orientations of the hydrogens bound to the cyclohexenyl ring in the major (half-chair) conformation I are in full agreement with the experimentally observed proton NMR coupling constants.

Introduction

Dibenzo[α,β]pyrene (DB[α,β]P*) is the most carcinogenic polycyclic aromatic hydrocarbon (PAH) presently known. Recent studies in which DB[α,β]P was applied to mouse skin or to rat mammary gland have shown that at low doses its tumor-initiating activity is significantly greater than that of benzo[a]pyrene or 7,12 dimethyl benz[a]anthracene (1,2). DB[α,β]P has been identified in river sediments (3), and in indoor (4) and outdoor (5) air samples, suggesting potential (eco)toxicological hazards. The mutagenic activity of DB[α,β]P is believed to be initiated by the formation of covalent adducts with DNA following enzymatic activation of the hydrocarbon. According to one mechanism, DB[α,β]P can be activated via a one-electron oxidation process (6) and bind covalently to the N3/N7 positions of adenine or to the N7/C8 positions of guanine residues (6,7). These reactions destabilize the glycosylic bond and lead to loss of the modified base (depurination). A second pathway involves the metabolic activation to diolepoxide (DE*) intermediates (8). Luch et al. (9) reported that the mutagenic activity of DB[α,β]PDE in Salmonella typhimurium and in Chinese hamster V79 cells was greater than any of the previously studied PAH diolepoxides. In mouse skin, (±)-syn-DB[α,β]P diolepoxide (in which the 11-hydroxyl and the 13,14-epoxide groups are cis) was found to be a more potent tumorigen than (±)-anti-DB[α,β]P diolepoxide (11-OH and 13,14-epoxide trans) (10). Recent studies by Cavalieri and co-workers have shown that both syn and anti diolepoxides can bind covalently to purine bases to form a mixture of stable and depurinating adducts. Reaction with the exocyclic amino groups of adenine or guanine leads to the formation of adducts that are stable under normal DNA isolation procedures (11,12), while binding at the N3 or N7 positions of adenine or the N7 position of guanine leads to depurination (7). Two depurinating adducts, syn-DB[α,β]PDE-14-N7 Ade (major) and anti-DB[α,β]PDE-14-N7 Gua (minor) were identified in the microsome-catalyzed reaction of DB[α,β]P with DNA (7). It has been suggested that depurination could be a major pathway in PAH-induced carcinogenesis (13).

Low-temperature fluorescence spectroscopy has proven to be a valuable tool for the characterization of both stable and depurinating DNA adducts from various PAHs (14). Fluorescence line-narrowing spectroscopy (FLNS) can be used for the fingerprint identification of closely related isomers (15–17), while the combination of FLNS and low-resolution fluorescence spectroscopy is useful for conformational analysis (18–20). When applying these techniques to the identification of depurinating adducts from DB[α,β]P, it was observed that different adducts derived from syn-DB[α,β]PDE yield fluorescence spectra with the (0,0) origin band located either near 382 nm, or near 389 nm, or with two origin bands located at these wavelengths (7). It was suggested that these phenomena could be due to the existence of multiple conformations.

In this paper we will describe the results of a more detailed spectroscopic and theoretical investigation into the conformational characteristics of these adducts. The compounds studied are the adducts from (±)-syn-DB[α,β]P diolepoxide to N7 or N3 of adenine or to N7 of guanine (see Figure 1 for molecular structures). For comparison the hydrolysis product syn-DB[α,β]P tetraol was studied as well. Theoretical calculations (molecular mechanics, dynamical simulations and semi-empirical calculations of electronic transitions) are used to interpret the experimental data. A thorough understanding of the conformational behavior will be helpful for the spectroscopic identification of depurinating adducts, but will also provide insight into the biological consequences of DNA adduct formation. The conformation of the diolepoxide inter-
mediate may be an important factor in determining whether adenine or guanine residues are the preferred targets of sterically crowded PAH diolepoxides (21,22). Furthermore, the conformational preferences of a stable adduct may determine how it is embedded in the DNA helix, and several authors have provided evidence that conformational effects strongly influence the mutagenic activity and manipulability of PAH-DNA lesions (23–25).

Materials and methods

Sample preparation

Molecular structures of the compounds investigated are shown in Figure 1. All samples contained racemic mixtures; the structures depicted correspond to derivatives of the 11R,12S,13R,14S diolepoxide. (+)-syn-DB[a,j]PDE-14-N7Ade, (±)-syn-DB[a,j]PDE-14-N7Gua and (±)-syn-DB[a,j]PDE-14-N3Ade, were obtained as part of our ongoing co-operation with the Eppley Institute of the University of Nebraska Medical Center (Dr Cavallero and Dr Rogan). The preparation of these adducts from (+)-syn-DB[a,j]PDE-14-N7Ade has been described by Li et al. (11, Li et al. submitted Chem. Res. Toxicol.). Proof of structure was obtained by means of NMR and tandem mass spectrometry. The stereochemistry of addition of these synthetic adducts had not been firmly established: NMR data showed that the N3Ade and N7Gua samples were in fact mixtures of cis- and trans-diastereoisomers, not separable by HPLC, while the N7Ade adduct actually appeared to be pure (Li et al. submitted Chem. Res. Toxicol.). Stereoselective assignment based on the spectroscopic and theoretical results obtained in this study will be discussed below. Tetrosil were obtained by hydrolysis of (+)-syn-DB[a,j]PDE-14-N7Ade; cis- and trans-isomers were separated by HPLC.

Two solvent matrices of different polarities were used for low-temperature spectroscopy: ethanol or water/glycerol 50:50 v/v. Ethanol was spectrophotometric grade from Aldrich; glycerol (ultra pure grade) was purchased from Spectro Chemical, Gardena, CA. Solutions (ca. 20 µl) were transferred to quartz tubes (2 mm i.d.) and sealed with a rubber septum. Concentrations were in the 10^{-5} to 5 × 10^{-6} M range. No spectral dependence on adduct concentration or cooling rate was observed.

Low temperature fluorescence spectroscopy

High-resolution FLN spectra (S_{T}−S_{0} excitation; T = 42 K) and low-resolution fluorescence spectra (S_{T}−S_{0} excitation, T = 77 K) were recorded using excitation source a Lambda Physik FL-2002 dye laser pumped by a Lambda Physik Lexera 100 XeCl excimer laser. For FLN spectroscopy many different excitation wavelengths were used, each revealing a portion of the S_{T} excited-state vibrational frequencies. Most low-resolution spectra were obtained using non-selective excitation at 308 nm (attenuated excimer laser). Samples were cooled in a glass cryostat with quartz optical windows. Fluorescence was dispersed by a McPherson 2016 1-m focal length monochromator, and detected by a Princeton Instruments IRY 1024x1024 intensified photodiode array. For time-resolved detection a Princeton Instruments FG-100 pulse generator was employed; the detector delay time and gate width were set to 45200 ns. For FLNS measurements the monochromator was equipped with a 1200 Gr/mm grating, providing an 18 nm spectral window at 0.1 nm resolution. For low-resolution spectroscopy a 150 Gr/mm grating was employed (150 nm window and 0.8 nm resolution).

**Fig. 1.** Molecular structures of trans-syn-DB[a,j]PDE-14-N3Ade and the three depurinizing adducts studied. The dihedral angles α and β will be used to describe the deviation from planarity in the fjord- and bay-regions, respectively. All samples contained racemic mixtures: the cis/trans stereochemistry of substitution at C-14 is discussed in the text.

Molecular modeling and theoretical calculations

**Molecular mechanics.** Conformational analyses of trans-syn-DB[a,j]PDE-14-N7Ade and trans-syn-DB[a,j]PDE-14-N7Gua and trans-syn-DB[a,j]PDE-14-N3Ade were carried out utilizing methods of molecular mechanics (MM), wherein energy calculations were performed with HyperChem's molecular modeling program (Release 3 for Windows^®). Hypercube Inc., Waterloo, Ontario, Canada). We have employed HyperChem's force field parameters describing an 'all atom' force field (MM+) for development of organic molecules, which is an extension of the MM2 method (26,27). Default parameters were used.

As starting structures we used two different model-built configurations in which the saturated ring was in a half-chair conformation and the substituent at C14 was in either an equatorial or an axial position. The Polak-Ribiere algorithm (the conjugate gradient method, in vacuo) was used for molecular mechanics optimization: the structures were refined until the r.m.s. gradient was less than 0.001 kcal/mol. In the above calculations the electrostatic contributions were evaluated from a set of bond dipole moments associated with polar bonds.

**Molecular dynamics.** In molecular dynamics simulations we used two different starting structures refined by geometry optimization as described above. 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. No constraints were used during high-temperature searches of the conformational space. Both structures were minimized and then subjected to 20 ps of molecular dynamics at various temperatures between 300 and 400 K. Starting and final temperature in a dynamic run was set to 0 K, and the heat and cool time was set to 5 ps; the step size was 0.0005 ps. At various timepoints during the simulation approximately 60 randomly selected structures were also annealed to 0 K and optimized. Those optimized structures were subsequently used as starting points for further calculations. All simulations were performed in vacuo. Three dihedral angles, defining the distortion in the bay-region (β), the fjord-region (α) and the conformation of the cyclohexenyl ring (C11-C12-C13-C14) were used as variables during the exploration of the conformational space. Optimization was restricted to the ground states, since only minor geometry changes between the S_{0} and S_{T} states of large PAH molecules are expected.

**Semi-empirical quantum mechanical calculations (0.0) transitions.** In order to explain the spectroscopically observed energy differences between the (0.0) origin bands of different adduct conformations, we used semi-empirical quantum mechanical calculations for the above determined low-energy conformations. We applied HyperChem's ZINDO/S method (an INDO method developed by Zerner's group), parameterized to reproduce UV-visible spectroscopic transitions using a configuration interaction (CI) treatment with 169 and 130 singly excited configurations for trans-syn-DB[a,j]PDE-14-N7Ade and trans-syn-DB[a,j]PDE-14-N7Gua, respectively. The CI space was truncated by considering only the eight lowest singly excited configurations. It was assumed that, in the first approximation, this approach would describe our systems with sufficient accuracy, since we were interested in the relative energy differences between the S_{0}→S_{T} electronic transitions rather than in absolute values. For UV spectra and orbital eigenvalues, a value of 1.267 for σ(28) and 0.385 for π(π) (29,30) overlap weighting factor were used.
Results

Low-temperature fluorescence spectroscopy

Low-resolution fluorescence spectra of the three synthetic adducts (and tetraol) from syn-DB[a,]PDE, measured at 77 K in two different solvent matrices, are presented in Figure 2. Unexpectedly, although the chemical differences between the three adducts occur several saturated bonds away from the benzo[e]pyrene chromophore, the spectra show remarkable differences even under low-resolution conditions. The N7Ade adduct (spectra 2c and 2d) shows its (0,0) origin band near 382 nm, while the first strong emission band of the N3Ade adduct (spectra 2g and 2h) appears near 389 nm (see also Table I). The N7Gua adduct (spectra 2e and 2f) shows emission maxima at both wavelengths. Especially in the case of the N7Gua adduct there is a clear spectral dependence on solvent composition: in ethanol the 382.5 nm peak and 389.3 nm peak are of comparable intensity, while in water/glycerol 50:50 the long-wavelength band gains in relative intensity. The same effect is observed for the two adenine adducts: the N3Ade adduct features a weak band at 382.5 nm in ethanol, which is not observed in the water/glycerol matrix. For the N7Ade adduct the same shift occurs, but the effect is masked by S0 vibronic emission bands and can only be observed under FLN conditions (see below). The low-resolution spectra of trans-syn-DB[a,]P tetraol (spectra 2a and 2b) are very similar to that of the N7Ade adduct in both solvent systems. Further low-resolution fluorescence studies showed that the relative intensities of the 382 and 389 bands also depend on excitation wavelength (spectra not shown), and it was established that the two bands constitute different origin bands. The intensity ratios of origin bands I and II did not depend on concentration, and both origin bands showed similar fluorescence lifetimes in both solvent systems (ca. 40 ns). For these reasons it was concluded that the occurrence of two distinct origin bands and the intensity ratio dependence on solvent composition could not be explained by assuming the samples to contain a mixture of two chemically distinct species. We postulated that the effect could be due to the existence of multiple (ground state) conformations that are in thermal equilibrium at room temperature, but that can be trapped separately during cooling to cryogenic temperatures. We then decided to carry out fluorescence line-narrowing spectroscopy and theoretical studies to investigate this phenomenon in further detail.

Fluorescence line-narrowing spectra obtained at 4.2 K are shown in Figure 3. In each case the excitation wavelength was chosen to optimally excite the S1 vibronic region 700–800 cm⁻¹ above each origin band. The sharpness of the zero-phonon lines (ZPLs) and their relative resistance to hole-burning served as additional evidence that the ZPLs in the 387–390 nm region are vibronically excited (0,0) bands and not (0,0)-excited ground state vibronic lines. Frame 3A shows the origin I region, employing vibronic excitation at 372.00 nm. The vibrational patterns observed for the three adducts (and also for trans-syn-DB[a,]P tetraol) reveal only minor differences, indicating that all four derivatives have the same stereochemical configuration. Frame 3B shows that the N7Ade adduct in water/glycerol (50:50) features a second set of vibronically excited (0,0) lines in the 387 nm region. These ZPLs were not observed when the sample was dissolved in ethanol or when the water/glycerol solution was five times diluted with ethanol. Interestingly, origin bands I and II both show ZPLs without significant phonon side bands, but the S1 vibrational frequencies are very different. Three major vibrational modes, 730, 751 and 798 cm⁻¹, are observed in the first origin region (spectrum 3b), while origin band II shows only two strong modes at 748 and 772 cm⁻¹ (spectrum

Table I. Fluorescence (0,0) origin bands of syn-DB[a,]PDE derivatives in ethanol and H2O/glycerol matrices; T = 77 K, λex = 308 nm

<table>
<thead>
<tr>
<th>Adduct or tetraol of syn-DB[a,]PDE</th>
<th>Wavelength of (0,0) band [nm]</th>
<th>Origin band I</th>
<th>Origin band II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>H2O/glycerol</td>
<td>Ethanol</td>
</tr>
<tr>
<td>trans-tetraol</td>
<td>382.2</td>
<td>382.7</td>
<td>*</td>
</tr>
<tr>
<td>-N7Ade</td>
<td>382.1</td>
<td>382.4</td>
<td>*</td>
</tr>
<tr>
<td>-N7Gua</td>
<td>382.5</td>
<td>382.5</td>
<td>389.3</td>
</tr>
<tr>
<td>-N3Ade</td>
<td>382.5</td>
<td>*</td>
<td>389.1</td>
</tr>
</tbody>
</table>

*Not observed in this solvent matrix, the relative population of a particular conformation is solvent dependent as demonstrated in Figure 2.
F.Ariese, G.J.-Small and R.Jankowiak

Origin Band I

Fluorescence Intensity

λ<sub>v</sub> = 372 nm

382 384 388 390
Wavelength (nm)

Origin Band II

Fluorescence Intensity

λ<sub>v</sub> = 378 nm

382 384 388 390
Wavelength (nm)

Fig. 3. High-resolution FLN spectra using vibronic excitation into the 700–800 cm<sup>-1</sup> region above each distinct (0,0) origin band. The solvent matrix was chosen to yield maximum intensity for the particular (0,0) band: ethanol for origin I (frame A) and water/glycerol 50:50 for origin II (frames B and C). Curves a and e, trans-syn-DB[a,\]PDE-14-N7Gua; curves b and f, syn-DB[a,\]PDE-14-N7Ade; curves c and g, syn-DB[a,\]PDE-14-N7Ade; curves d and h, syn-DB[a,\]PDE-14-N3Ade. Peaks are labeled with their excited state vibrational frequencies; T = 4.2 K. The minor peaks indicated by * do not belong to the origin band II, but correspond to a 390/cm ground state vibration (progression of the origin multiplet).

3f). This indicates that the red-shift of origin band II could not be solely the result of stacking interactions between the base and the aromatic moiety, since recent results on benzo[a]pyrene diolepoxide adducts showed that such stacked conformations lead to moderate red-shifts and strong electron-phonon coupling, but not to significant changes in vibrational frequencies (20). The FLN results thus indicate that, although stacking interactions could play a role, the compound must also undergo other conformational changes that have a more direct impact on the vibrational modes of the chromophore.

Also for the N7Gua and N3Ade adducts we observed relatively sharp ZPLs in the second origin region around 389 nm (see Figure 3C). Since for these two adducts the origin band II is further red-shifted than in the case of the N7Ade adduct we used 378.00 nm excitation in order to probe the same vibronic region. The vibronic frequency patterns of spectra 3g and 3h are again very similar to that observed in the origin II band of the N7Ade adduct (spectrum 3f). In the case of trans-syn-DB[a,\]PDE-14-N7Gua, an extremely weak origin band II can be observed in the 387 nm region in the water/glycerol matrix, but not in ethanol. The FLN lines are even weaker than in the case of the N7Ade adduct, but the vibrational frequencies are identical (see Figure 3B).

The FLN spectra shown in Figure 4 for the N7Gua adduct in two different solvent systems serve as a high-resolution illustration of the solvent effect. In water/glycerol 50/50 the ZPLs in the origin I region are significantly weaker than in ethanol and origin band II shows a small red-shift, but the change in solvent does not affect the vibrational frequencies observed within each origin band. The latter is in agreement with previous observations for benzo[a]pyrene diolepoxide adducts (20).

Molecular modeling and theoretical calculations

Simulations of structural conformations. As demonstrated above by the low-temperature fluorescence spectra, the adducts of syn-DB[a,\]PDE may exist in two conformations, and sometimes even a mixture of conformations is trapped. The conformational equilibrium is strongly solvent-dependent (see Figures 2 and 4). In order to understand these phenomena, a theoretical investigation was started using molecular mechanics and molecular dynamics simulations. Dynamical simulation studies were carried out at temperatures between 300 and 400 K; local minima in the potential energy surface were searched for by simulated rapid cooling of approximately 60 high-temperature structures and subsequent optimization. Several different starting structures were used: first the two half-chair structures obtained from molecular mechanics, but we also used the optimized structures obtained during simulated annealing as starting points for subsequent simulations. However, at higher temperatures the same conformational equilibrium was reached, regardless of the starting conformation. Our calculations focused on syn-DB[a,\]PDE-14-N7Ade, one of the major depurinating adducts formed in vitro (7). In the first instance calculations were carried out for both cis and trans addition products. Molecular simulations carried out for the cis-N7Ade adduct could not reproduce the experimentally observed occurrence of dual conformations: a single structure was observed in which the cyclohexenyl ring adopts a distorted half-chair conformation with the base in a pseudo-axial position (not shown). For trans-syn-DB[a,\]PDE-14-N7Ade two unique structures were observed that correspond to the lowest identified energy minima (see Figure 5). As the result of steric crowding, there is a severe distortion from planarity in the fjord-region in both cases. In conformation I (frame 5A) the distal aromatic ring is bent towards the adenine moiety;
the cyclohexenyl ring adopts a boat-type conformation with the adducted base in a pseudo-axial position. In this 'open' structure no positive interaction between the base and the aromatic system is possible. In the 'folded' conformation II the adenine moiety is in a pseudo-equatorial position, partially stacked over the distal ring that is now bent in the opposite direction. In this conformation the cyclohexenyl ring adopts a half-chair structure (see frame 5B), very similar to one of the starting structures. Calculated minimum energies (in isolation, no solvent) of the two conformers are listed in Table II. Interconversions at temperatures > 300 K indicated that these conformations are in equilibrium on a picosecond timescale (also the spectroscopic behavior in different solvents shows that the two conformations interconvert readily at room temperatures). During the simulations some higher-energy conformations were also observed, for instance a half-boat structure with the adenine moiety in a pseudo-equatorial position and the distal ring bent away from the base. However, this structure could not be trapped separately and would convert to conformation II during simulated cooling. Also the second starting structure (half-chair with base axial) could be observed at higher temperature, but was not trapped during annealing. Since we did not find actual local minima for those higher-energy structures we did not attempt to estimate the corresponding energy levels.

For trans-syn-DB[a]P-tetraol very similar conformations were obtained, as depicted in Figure 6. Molecular dynamics simulations showed that the saturated ring can adopt a half-boat (frame 6A) or half-chair-type structure (frame 6B). Apparently, the presence of the purine substituent is not a prerequisite for the occurrence of these two distinct conformations. The interconversion occurs much more rapidly than in the case of the N7Ade adduct (lower barrier) but again both conformations can be trapped at cryogenic temperatures. Calculated ground state energies for trans-syn-DB[a]P-tetraol are -1.0 ± 0.1 and -3.5 ± 0.2 kcal/mol for conformers I and II, respectively.

Analysis of the dihedral angles for the proton pairs 11-12, 12-13, and 13-14 of the cyclohexenyl ring of conformers I and II (see Figure 5) may be used to interpret the vicinal proton–proton coupling constants observed experimentally by 1H NMR. The dihedral angles calculated for the two conformations of trans-syn-DB[a]PDE-14-N7Ade are listed in Table III. From these dihedral angles the corresponding coupling constants can be estimated using Karplus' relations (31). For the N7Ade adduct in conformation I (half-boat) the dihedral angles predict the following set of \( J_i \) values: \( J_{11,12} = \) large, \( J_{12,13} = \) small and \( J_{13,14} = \) small. For the half-chair conformation II the predicted coupling constants are large, large and large, since the four protons are all-trans in pseudo-axial positions. As shown in Table III the proton–proton coupling constants obtained for conformation I agree very well with those observed experimentally (Li et al. submitted Chem. Res. Toxicol.) and with literature data compiled by Jerina and co-workers (32) for other trans adducts to syn-diolepoxides of sterically hindered PAHs. These findings provide additional evidence that the N7Ade adduct is formed by trans-addition to the epoxide, and that the half-boat conformation is the major conformation at ambient temperature. In contrast, coupling constants predicted for the cis-adduct of syn-DB[a]PDE to N7-Adenine are: \( J_{11,12} = \) small, \( J_{12,13} = \) large and \( J_{13,14} = \) small (distorted half-chair conformation with the base in a pseudo-axial position), in disagreement with the experimental values in Table III.

![Fig. 5. Optimized 0 K ground state structures of trans-syn-DB[a]PDE-14-N7Ade obtained after simulated annealing. Conformer I is shown in frame A, conformer II is presented in frame B. Complete molecular structures are shown without hydrogens and double bonds for clarity. The insets show the conformation of the cyclohexenyl ring (half-boat versus half-chair) in more detail; R = N7Ade.](image)

Table II. Calculated and experimentally observed (0.0) origin bands for trans-syn-DB[a]PDE-14-N7Ade

<table>
<thead>
<tr>
<th>Conformer</th>
<th>Energy (kcal/mol)*</th>
<th>( \lambda_{\text{calc}} ) (0.0) (nm)</th>
<th>Oscillator strength*</th>
<th>( \lambda_{\text{obs}} ) (0.0) (nm)</th>
<th>( \Delta\lambda_{\text{calc}} ) (nm)*</th>
<th>( \Delta\lambda_{\text{obs}} ) (nm)*</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>14.60</td>
<td>380.3</td>
<td>0.012</td>
<td>382.4</td>
<td>5.0</td>
<td>4.6</td>
<td>half-boat (major)</td>
</tr>
<tr>
<td>II</td>
<td>9.96</td>
<td>385.3</td>
<td>0.007</td>
<td>~387</td>
<td></td>
<td></td>
<td>half-chair (minor)</td>
</tr>
</tbody>
</table>

*Ground state energy minimum in isolation (no solvent).
*Transition only weakly allowed for both conformers (see text).
*Wavelength difference between (0,0) transitions calculated for conformations I and II.
*Wavelength difference observed between fluorescence origin bands I and II.

Syn-dibenzo[a]pyrene diolepoxide, conformational studies
Calculation of $0-0$ electronic transitions. In order to interpret the spectroscopically observed occurrence of two distinct $(0,0)$ transitions, we carried out semi-empirical quantum mechanical calculations for the structures obtained from molecular dynamics simulations. The calculations were performed with a relatively simple CI method including only singly excited configurations. We calculated the difference between the ground and the first excited electronic state for the half-boat and half-chair conformations and compared the results with the spectroscopic data. The results obtained for $trans$-$syn$-$DB[a,\,l]P$ tetraol are summarized in Table II. For the spectral shifts of the $(0,0)$ origin bands a good correlation between the calculated and experimental values was obtained. For example, the calculated energy difference (5.0 nm) between the two $(0,0)$ transitions of conformers I and II is in very good agreement with the value of 4.6 nm obtained spectroscopically (see Table II). For $trans$-$syn$-$DB[a,\,l]P$ tetraol the calculated $(0,0)$ transition wavelengths are $381.4 \pm 0.3$ nm and $384.0 \pm 0.2$ nm for the half-boat and the half-chair conformations, respectively. The very small oscillator strengths for adducts and tetraols in both conformations are in good agreement with the weakly allowed first electronic transition of benzo[e]pyrene, which is the aromatic moiety in $DB[a,\,l]P$ diolepoxide derivatives. Overall, the absolute values of the $(0,0)$ transitions calculated in vacuum are slightly blue-shifted compared to the origin bands observed in solution, but based on the relative $(0,0)$ energies calculated for the two conformers an assignment of the experimentally observed origin bands can be made. We conclude that the blue-shifted $(0,0)$ transition of the major conformation of $trans$-$syn$-$DB[a,\,l]P$DE-14-N7Ade (observed at 382.4 nm, see Figure 2) corresponds to that calculated for the half-boat conformer, while the red-shifted origin band of the minor conformer, observed at ca. 387 nm, corresponds to the half-chair conformer II.

Ground state structural distortions. In this section the intramolecular steric interactions in $DB[a,\,l]P$ derivatives and the resulting non-planarity of the aromatic skeleton are briefly addressed. The ground state distortion of the aromatic moiety, caused by steric crowding in the fjord-region, is clearly demonstrated in Figures 5 and 6. We introduce the dihedral angles $\alpha$ and $\beta$ (see Figure 1) to describe the deviation from planarity in the fjord and bay-regions, respectively. As shown in Table IV only a minor distortion from planarity is observed in the $\beta$-dihedral angle (~2°), while large twisting is observed in the fjord-region (~25°). Our calculations indicate that for the half-boat conformations (I) the extent of distortion is identical for $trans$-$syn$-$DB[a,\,l]P$DE-14-N7Ade and for $trans$-$syn$-$DB[a,\,l]P$ tetraol, but in the case of the half-chair conformer II, in which the N7Ade or hydroxyl substituent at C-14 is in a pseudo-equatorial position, the deviation from planarity is larger for the N7Ade adduct, presumably the direct result of its larger size. Note that in conformations I and II the distortion is manifested in opposite directions, with the carbon atom C-1 bent away from the pseudo-equatorial substituent at C-14. Calculations predict a similar preference for the direction of distortion for benzo[c]phenanthrene diolepoxides (33). Lewis-Bevan et al. suggested that electrostatic interactions could be responsible for this effect (33), but steric factors could also play a role: the distortion of the aromatic system necessary to obtain sufficient clearance in the bay-region is smallest if the distal ring is bent away from the equatorial substituent, towards the axial substituent. Both effects may be important in determining which structure is thermodynamically favored. It was apparent that the crossing of the distal ring through the plane of the aromatic moiety ($\alpha=0$) constitutes the major energy barrier between conformations I and II. During simulations such crossings were relatively rare, while in the same timespan the cyclohexenyl ring would frequently change conformations. When the system was annealed with the distal ring bent towards the base (upwards in Figure 5A), the system would always adopt the boat-type conformation I. When, on the other hand, the simulated cooling started when the distal ring was bent away from the base (down in Figure 5B), the cyclohexenyl ring would always adopt a chair-type conformation with the base in a pseudo-equatorial position, stacked over the distal ring.

![Fig. 6. Optimized 0 K ground state structures of $trans$-$syn$-$DB[a,\,l]P$ tetraol obtained after simulated annealing. Conformer I is shown in frame A; conformer II is presented in frame B. Hydrogens and double bonds are omitted for clarity.](image-url)
interaction is a major parameter in the relative stability of the conformation. It agrees with our assumption that J1-7 stacking.

The fact that the tetraol exists almost exclusively in the ‘open’ conformations suggests an accurate picture of a system in solution. An alternative explanation could be related to the shape of the potential energy surface: if we assume that conformation II corresponds to a steep and narrow minimum, while conformation I is characterized by a very broad, shallow local minimum, the latter conformation would be statistically more populated at higher temperatures. Visualization of the simulations and simulated cooling at various randomly selected timepoints showed that most of the time the conformation of the adduct resembles or converts to conformation I.

The conformational equilibrium was found to shift towards the folded conformation II in a more polar solvent. This phenomenon can be explained by taking into account the poorer solvation of the aromatic moiety in water/glycerol and thus a stronger tendency towards (intramolecular) complexation. Unfortunately, at this time we were not able to perform conformational simulations in solution to study this effect in more detail.

Our theoretical investigations also point at a significant deviation from planarity for the DB[a,1]P derivatives studied. Similar distortions of the aromatic moiety have been reported for other PAHs featuring a fjord-region or a methyl-substituted bay-region. For example, a 22° distortion was measured for 7,12 dimethyl benz[a]anthracene (36), while the bay-region methyl group of 3,6-dimethylcholanthrene distorts the aromatic moiety from an almost planar structure to one with an angle of 16° between the outer rings (37). Molecular orbital theoretical calculations were applied to predict distortions for methyl-substituted bay-region PAHs (38) and for bay- and fjord-region diolepoxides (33). Interestingly, methyl substitution in the bay-region is typically associated with increased tumor-initiating activity (39). Many fjord-region PAHs also react extensively with DNA and are among the strongest PAH mutagens (40,41). There are several ways in which steric crowding can influence DNA-adduct formation and subsequent biological processes. The reduced ability of the aromatic moiety to delocalize a developing cationic charge will cause the diolepoxide derivative to be more resistant to hydrolysis, this way increasing the potential adduct yield (32,42).

Non-planarity and the preferred orientation of the epoxide and hydroxyl groups will also influence the alignment of the reactive diolepoxides with the DNA helix. These steric effects may thus influence the relative reactivities of syn- and anti-diolepoxides, the relative reaction rates with ring nitrogens and exocyclic amino groups, the cis-trans stereochemistry of addition and the ratio of adenine versus guanine modifications. It has been suggested (22,42) that non-planarity in benzo[c]phenanthrene diolepoxides may be associated with the observed higher chemical reactivities with adenine residues than with guanines. In general, a relatively high reactivity towards adenine residues appears to be associated with a high tumorigenic potency (13,40). DB[a,1]P appears to follow this general rule: recent results show that upon microsome activation the

**Table IV. Deformation of the aromatic moiety calculated for trans-syn-DB[a,1]PDE-14-N7Ade and trans-syn-DB[a,1]P tetraol. α and β are the dihedral angles (in degrees) describing the deviation from planarity in the fjord- and bay-regions, respectively (see Figure 1)**

<table>
<thead>
<tr>
<th>Dihedral angles</th>
<th>trans-syn-DB[a,1]PDE-14-N7Ade</th>
<th>trans-syn-DB[a,1]P-tetraol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conformer I</td>
<td>Conformer I</td>
<td>Conformer I</td>
</tr>
<tr>
<td>α</td>
<td>-25.3</td>
<td>-24.9</td>
</tr>
<tr>
<td>β</td>
<td>-0.9</td>
<td>-2.0</td>
</tr>
</tbody>
</table>

**Discussion**

We have found that depurinating adducts from syn-DB[a,1]P diolepoxide may exist in two different conformations which are in thermal equilibrium at room temperature, but can be trapped at cryogenic temperatures. Also higher-energy conformations that could contribute to the conformational equilibrium at room temperature were observed during dynamical simulations, but those minor conformations could not be trapped in simulated annealing experiments and were not investigated in detail. The two structures that correspond to local energy minima suffice to fully explain the low-temperature fluorescence and room temperature NMR spectra.

Theoretically calculated (0,0) origin bands are in fairly good agreement with experimental data. The cyclohexenyl ring of trans-syn-DB[a,1]PDE-14-N7Ade adopts primarily a half-boat configuration with the added base in a pseudo-axial position. This structure is in full agreement with the NMR coupling constants for the protons of the cyclohexenyl ring (see Table III). Very recently, based on NMR data, a similar structure was proposed by Luch as the major conformation for trans-syn-DB[a,1]P tetraol (34). Given the similarity of the FLN spectra of this tetraol and of the N7Ade adduct, the stereochemistry and conformational preference of these two compounds must be identical. The boat-type conformation is rather unusual, since in most syn- and anti-diolepoxide adducts studied to date, the cyclohexenyl ring adopts a half-chair conformation with the base in a pseudo-axial position (32). However, for trans-syn adducts such a structure is highly unfavorable due to 11-13 and 12-14 diaxial interactions. Instead, conformation II, with all substituents pseudo-equatorial, is observed for bay-region diolepoxide adducts with only moderate steric hindrance (32) and as shown in this report it is also the preferred conformation of the N7Gua and the N3Ade adduct. Most probably π-π interaction contributes to this conformational preference, but it is yet unclear why the conformational equilibrium of the N7Gua would be so much different from that of the N7Ade adduct. Conformation I appears to be more favorable in severely hindered fjord-region compounds such as benzo[c]phenanthrene diolepoxide adducts (35) and we found this boat-type structure to be the major conformation of syn-DB[a,1]PDE-14-N7Ade and of trans-syn-DB[a,1]P tetraol. The fact that the tetraol exists almost exclusively in the ‘open’ conformation I agrees with our assumption that π-π stacking interaction is a major parameter in the relative stability of the ‘folded’ conformation II.

The fluorescence and NMR data indicate that the N7Ade adduct exists predominantly in the ‘open’ conformation I. However, this does not agree with the fact that a lower potential energy was calculated for the stacked conformation II (see Table II). This discrepancy could of course be due to the fact that calculations carried out in vacuum do not always yield an accurate picture of a system in solution.

#### Table IV. Deformation of the aromatic moiety calculated for trans-syn-DB[a,1]PDE-14-N7Ade and trans-syn-DB[a,1]P tetraol. α and β are the dihedral angles (in degrees) describing the deviation from planarity in the fjord- and bay-regions, respectively (see Figure 1)
major DNA adducts are formed with adenine, with trans-syn-DB[a]PDE-14-N7Ade accounting for 31% of the total adducts (7). Conformational factors are also important after the adduct has been formed. In the case of stable adduct formation, deviations from planarity and the preferred conformation of the cyclohexenyl ring will influence the way in which these adducts will be embedded in the DNA helix, which in turn will influence their biological effects (23,24) and recognition by repair enzymes (25,43). The results obtained in this study will not only be important for further spectroscopic characterization of DB[a,P] depurinating adducts, but they will also help us unravel the even more complex conformational behavior of stable adducts. We believe that conformational studies will prove increasingly important to our understanding of the carcinogenic activity of PAH diolepoxides.

Acknowledgements

Thanks are due to Drs K.-M.Lu and E.L.Cavaleri for providing us with the adduct samples and the corresponding proton NMR data. This research project was supported by the National Institute of Health, grant no. PO1 CA9210-05. Partial support to R.J. and G.J.S. by the Office of Health and Environmental Research of the US Department of Energy is also acknowledged The Ames Laboratory is operated for the US Department of Energy by Iowa State University under contract no. W-7405ENG-82.

References


Received on July 12, 1995: revised on December 18, 1995; accepted on December 19, 1995