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# Linear dichroism properties and orientations of different ultraviolet transition moments of benzo[*a*]pyrene derivatives bound noncovalently and covalently to DNA

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Linear dichroism and absorption methods are used to study the orientations of transition moments of absorption bands of polycyclic aromatic epoxide derivatives which overlap with those of the DNA band in the 240–300 nm region. Both the short and long axes of the pyrene residues of 1-oxiranylpyrene (1-OP) and the (+) and (−) enantiomers of *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) noncovalently bound to double-stranded native DNA are oriented approximately perpendicular to the axis of the DNA helix, consistent with intercalative modes of binding. The covalent binding of these three epoxide derivatives to DNA is accompanied by reorientations of both the short and long axes of the pyrene residues. Covalent adducts derived from the highly mutagenic (+)-*anti*-BPDE are characterized by tilts of the short axis within 35° or less, and of the long axis by more than 60–80°, with respect to the planes of the DNA bases. In the adducts derived from the binding of the less mutagenic (−)-*anti*-BPDE and 1-OP epoxide derivatives to DNA, the long axes of the pyrenyl rings are predominantly oriented within 25° of the planes of the DNA bases; however, in the case of the (−) enantiomer of BPDE, there is significant heterogeneity of conformations. In the case of the 1-OP covalent DNA adducts, the short axis of the pyrene ring system is tilted away from the planes of the DNA bases, and the pyrene ring system is not intercalated between DNA base-pairs as in the noncovalent complexes. The stereochemical properties of the saturated 7,8,9,10-ring in BPDE, or the lack of the 7 and 8 carbon atoms in 1-OP, do not seem to affect noncovalent intercalative complex formation which, most likely, is influenced mainly by the flat pyrenyl residues. These structural features, however, strongly influence the conformations of the covalent adducts, which in turn may be responsible for the differences in the mutagenic activities of these molecules.

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are metabolically converted into reactive epoxide and other oxygenated derivatives by a series of microsomal enzymes in the cell [1–3]. The covalent binding of these reactive intermediates to nucleic acids is believed to be an important step in the initiation of the carcinogenic process and in mutagenesis [4–7]. The conformational properties of the PAH residues covalently bound to DNA are believed to play an important role in initiating these processes [4,7,8]. Linear dichroism (LD) techniques have been employed extensively to study the orientations of the aromatic residues of various PAH epoxide derivatives bound both noncovalently and covalently to DNA (see ref. 9 for a review).

The binding to DNA of the various stereoisomers of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, the ultimate mutagenic and

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tumorigenic metabolite of benzo[*a*]pyrene, can be easily studied by LD and other optical techniques because of the prominent pyrene-like absorption band in the 300–360 nm region of the spectrum. This band system, designated as $^1L_a$ in the Platt notation [10], is polarized within the plane and along the long axis ($z$) of the pyrenyl ring system [10], while the $^1B_b$ transition at 274–278 nm is polarized along the short, or $y$-axis.

In most previous reports, only the orientation of the $z$-axis was defined [9]. However, using signal averaging and computer-based subtraction techniques, it has now become possible to investigate the absorption and LD characteristics of transitions of PAH chromophores which overlap with those of the DNA bases below 300 nm [11–13].

In this work, the orientations of both the $z$- and $y$-polarized transitions of the pyrenyl residues in noncovalent complexes and covalent adducts derived from the physical and chemical binding to DNA of (±)-1-oxiranylpyrene (1-OP) and the (+) and (−) stereoisomers of *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-BPDE, fig. 1), are compared. The two enantiomers of BPDE were selected for study because of their differences in chemical reactivities and biological activities [2,4–7]. The epoxide 1-OP is of interest because it resembles BPDE, but lacks the 7,8 carbon atoms and the OH-substituents at these positions, and is less mutagenic than BPDE [5]. The overall goal of these and other studies [9] is to search for correlations between the structural properties of PAH epoxide metabolites, their reactivities with DNA, and the structural properties of the adducts formed which may influence DNA repair processes and replication in vivo. Another goal of these studies was to characterize the changes in orientation of both the short and long axes of the planar pyrenyl residue as the noncovalently complexed molecules react chemically with the DNA bases to form the more persistent and thus biologically more significant covalent adducts.

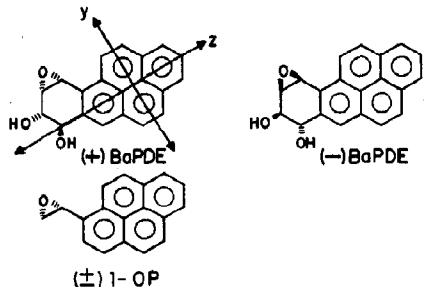


Fig. 1. Structures of (+)-*anti*-BPDE, (−)-*anti*-BPDE and 1-OP. The orientations of the long-axis ($z$) and short-axis ($y$) transition moments of the aromatic pyrenyl residue are also shown.

A more detailed investigation of the conformations of the BPDE stereoisomer-DNA complexes was prompted by our recent observation that noncovalent 3-methylcholanthrene diol epoxide-DNA complexes are not intercalative in nature [12]. However, there is a significant body of evidence that metabolites of benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons do indeed form noncovalent intercalation complexes with DNA [14]. These conclusions are confirmed here in the case of (+)- and (−)-*anti*-BPDE and 1-OP.

## 2. Materials and methods

Racemic 1-oxiranylpyrene was synthesized according to previously described methods [15]. Both (+)-*anti*-BPDE and (−)-*anti*-BPDE were purchased from the National Cancer Institute Chemical Carcinogen Reference Standard Repository (lot nos. 83-344-49-5 and 83-344-99, respectively). Calf thymus DNA (Worthington Chemicals, Freehold, NJ) was prepared and treated with BPDE or 1-OP as described previously [16–19]. All concentrations were determined spectrophotometrically using 6700 (at 258 nm) for DNA, and 29000 $M^{-1}$ $cm^{-1}$ (342–344 nm) for BPDE and 1-OP [17]. A Perkin-Elmer 320 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT), or a Hewlett-Packard diode array spectrophotometer (model 8451, Hewlett-Packard Corp., Corvallis, OR) were used in all absorption studies. The LD spectra were determined by orienting DNA in the hydrodynamic flow gradient of a Couette cell, and by measuring the absorbance with linearly polarized light using a specially constructed system described elsewhere [20]. LD difference spectra were obtained by first recording the spectra of

the epoxide-DNA physical complexes or covalent adducts, and subsequently subtracting those of unmodified DNA, using a Data 6000 digital waveform analyzer (Data Precision Analogics Corp., Medford, MA) interfaced with an IBM computer. Difference absorption spectra were determined in an analogous manner utilizing the commercially available spectrophotometers.

The LD signal is defined as

$$LD = A_{\parallel} - A_{\perp} \quad (1)$$

where $A_{\parallel}$ and $A_{\perp}$ denote absorbance measured with the polarization vector of the incident light beam oriented parallel and perpendicular, respectively, relative to the flow direction.

Stock solutions of epoxides were prepared in THF at concentrations of $10^{-3}$ M. Aliquots of these solutions were added to aqueous solutions of DNA in 5 mM sodium cacodylate buffer (pH 7.1). The final concentration of THF did not exceed 1% (v/v). For physical complex formation, DNA solutions were brought to pH $8.60 \pm 0.02$ by adding a dilute solution of NaOH in order to retard the rate of reaction of the epoxides [16]. LD spectra were then recorded within 2 min after mixing in order to minimize the extent of reaction of the epoxides.

The covalent BPDE or 1-OP-DNA adducts were prepared by allowing predetermined amounts of epoxide molecules to react with the DNA at room temperature [17,18]. The tetraol or other hydrolysis products were removed by repeated ether extraction and/or exhaustive dialysis. The level of covalent modification was estimated spectrophotometrically [17,21], and was of the order of one PAH residue per 100 DNA bases.

## 3. Results and discussion

### *3.1. Absorption spectra of BPDE and 1-OP in buffer solution*

The absorption spectra of 1-OP and (+)-*anti*-BPDE are shown in fig. 2. Above 300 nm, the absorption spectrum of BPDE is red-shifted by 2 nm with respect to that of 1-OP. The wavelength

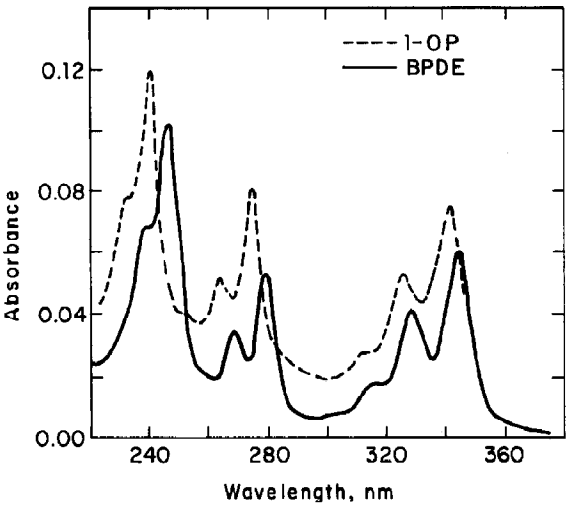


Fig. 2. Absorption spectra of (+)-*anti*-BPDE (2 μM) and 1-OP (2.6 μM) in aqueous buffer solution.

positions of the maxima are summarized in table 1, and compared to those of the noncovalent DNA complexes and covalent DNA adducts. The polarizations of the different absorption bands are also indicated.

Table 1

Absorption maxima (nm) of free, and noncovalently and covalently DNA-bound epoxides

| Polarization [a] | (+)- and (−)-*anti*-BPDE | | (+)-*anti*-BPDE (Covalent) | (−)-*anti*-BPDE (Covalent) |
|---|---|---|---|---|
| | (Buffer) | (Non-covalent) | | |
| *z* | 344 | 354 | 346 | 354 |
| *z* | 328 | 338 | 331 | 337 |
| *y* | 278 | 286 | 282 | 285 |
| *z* and *y* | 268 | 278 | 270 | 272 |
| *z* dominant, some *y* | 246 | 246 | 250 | 242 |
| 1-OP | | | | |
| *z* | 342 | 350 | 345 | |
| *z* | 326 | 336 | 329 | |
| *y* | 274 | 282 | 277 | |
| *z* and *y* | 264 | 267 | 266 | |
| *z* dominant, some *y* | 241 | 243 | 242 | |

[a] Ref. 1, p. 358. It is assumed that the directions of the transition moments in BPDE and 1-OP are the same as those in pyrene.

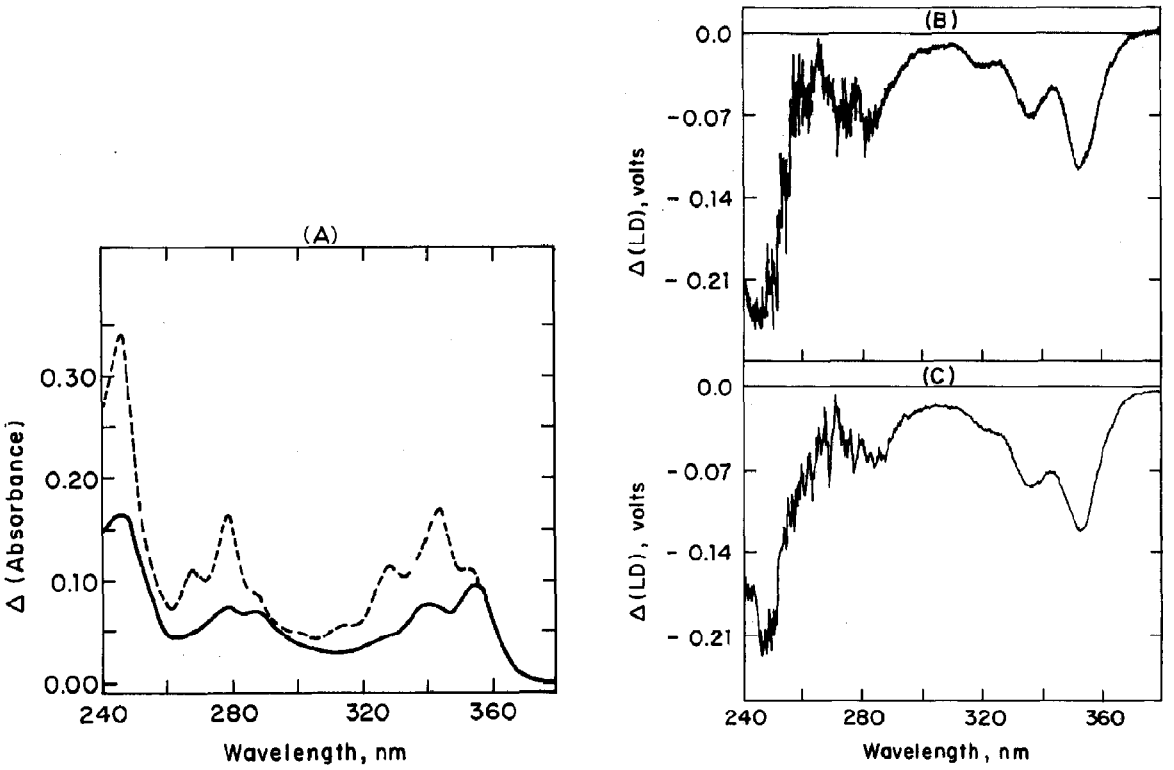


Fig. 3. Difference absorbance (eq. 4) and difference LD spectra ($LD_{complex} - LD_{DNA}$) of noncovalent BPDE-DNA complexes; [BPDE] = 8.6 μM, [DNA] = 0.15 mM (pH 8.6). (A) (- - - - -) Contribution of free and noncovalently bound (+)-*anti*-BPDE to the absorbance of aqueous BPDE-DNA mixtures. (———) Absorption spectrum of noncovalently bound (+)-*anti*-BPDE (see text; estimated concentration of bound BPDE: 5.7 μM). Note: analogous results are obtained with (−)-*anti*-BPDE. (B, C) Difference LD spectra of (+)-*anti*-BPDE- and (−)-*anti*-BPDE-DNA noncovalent complexes, respectively. Before the addition of BPDE, the LD signal of the DNA at 258 nm was −3.7 V; thus, the difference LD signals are less than 5% of the full DNA signal at 258 nm.

When PAH diol epoxide molecules are added to aqueous solutions of DNA, noncovalent complexes are formed on time scales of less than a few milliseconds [22]. The 1-OP [17] and BPDE [22–24] complexes are characterized by red shifts in the $^1L_a$ absorption band of about 10 nm, and the LD signals are negative in sign, indicative of a tilt of the *z*-axis of 35° or less with respect to the planes of the DNA bases [16–18].

Conformations in which there are significant pyrenyl residue-DNA base stacking interactions have been designated as site I-type [9,25]; in contrast, those in which the long or *z*-axes are tilted closer to the normals of the planes of the base-pairs and the pyrenyl residues are solvent-exposed [26–29] have been designated as site II. It is convenient to utilize these approximate designations to distinguish between different types of complexes or adducts.

### 3.2. Noncovalent complexes

#### 3.2.1. Absorption spectra

The absorption spectrum of a solution of (+)-*anti*-BPDE in DNA, referenced against a DNA solution of exactly the same DNA concentration, is shown in fig. 3A (dashed line). This difference absorption spectrum, $\Delta A$, is due to both free and noncovalently bound BPDE molecules.

The absorption spectrum of the physically bound BPDE molecules, $A_{complex}$, can be obtained

by subtracting the contribution of free BPDE molecules, $A_{free}$, from $\Delta A$:

$$A_{complex} = \Delta A - A_{free} \quad (2)$$

where

$$A_{free} = (1 - X) A_{X=0} \quad (3)$$

In eq. 3, $X$ represents the fraction of noncovalently bound BPDE molecules, and $A_{X=0}$ designates the absorption spectrum of BPDE molecules in the absence of DNA. The fraction of noncovalently bound molecules can be calculated at any given DNA concentration (expressed in terms of moles of nucleotides, [DNA]), by the following expression:

$$X = \frac{K[\mathrm{DNA}]}{1 + K[\mathrm{DNA}]} \quad (4)$$

where $K$ is the noncovalent association constant ($K = 12000\ \mathrm{M}^{-1}$ [20]); this equation is based on a simple binding equilibrium and is valid as long as the number of DNA-binding sites greatly exceeds the BPDE concentration. In the experiments of fig. 3, [DNA] = 0.15 mM and [BPDE] = 8.6 μM, thus giving $X = 0.64$. The absorption spectrum of free molecules was then calculated from the known values of $A_{X=0}$ for an 8.6 μM solution of BPDE in buffer. An absorption spectrum of noncovalent BPDE-DNA complexes thus obtained via eqs. 2–4, is shown in fig. 3A (solid line). An analogous absorption spectrum is obtained in the case of (−)-*anti*-BPDE (data not shown).

The absorption spectrum of 1-OP molecules noncovalently bound to DNA, and obtained in the same manner as that described for (+)-*anti*-BPDE, is shown in fig. 4A. The association constants of 1-OP and BPDE are known to be the same [17], and a value of $K = 12000\ \mathrm{M}^{-1}$ was used to estimate $A_{free}$, and thus the absorption spectrum of physically bound 1-OP molecules (fig. 4A). It is interesting to note that the *y*-polarized $^1B_b$ transition in the 270–290 nm region is red-shifted by 8 nm in both the 1-OP- and BPDE-DNA complexes; this is quite similar to the 10 nm red shift of the $^1L_a$, 310–360 nm band system. In contrast, there is no shift in the $^1B_a$ absorption maximum at 241–246 nm (table 1).

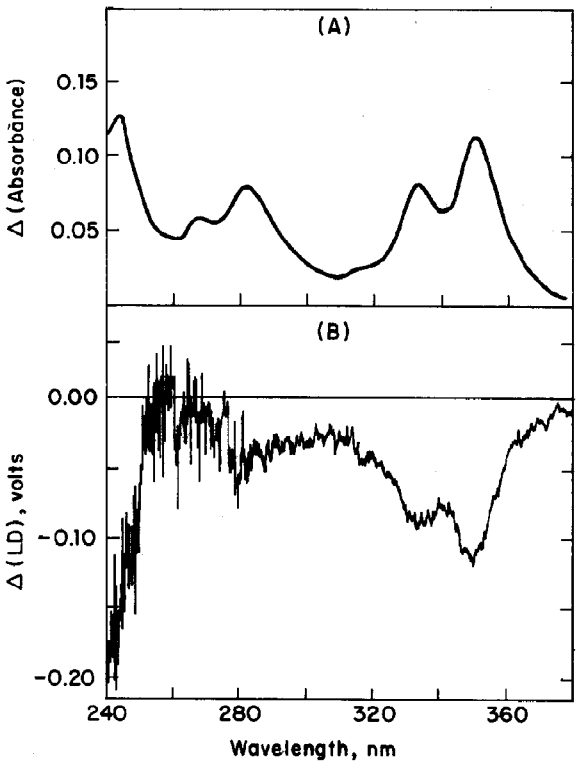


Fig. 4. Difference absorption and difference LD signal of 1-OP-DNA noncovalent complexes (pH 8.6). (A) Absorption spectrum of 1-OP noncovalently bound to DNA, determined as described in the text and in fig. 3A. (B) Difference LD spectrum. The value of the LD signal at 258 nm before the addition of 1-OP was −7.8 V. [DNA] = 0.16 mM, [1-OP] = 3.4 μM.

### 3.2.2. LD spectra

The signs and magnitudes of the LD signals are in general a function of the degree of alignment ($F(G)$) of the DNA molecules in the flow gradient $G$ ($0 < F(G) < 1.0$), the absorbance $A_{complex}$ (free molecules do not contribute to the LD spectra), and the angle of inclination, $\theta$, between the transition moment and the axis of orientation (the flow direction in this case). The relationship between these factors is given by [30]:

$$\mathrm{LD} = (3/2) A_{complex} (3 \cos^2\theta - 1) F(G) \quad (5)$$

In the case of unmodified DNA, $\theta$ is approx. 90° and thus the LD signal is negative in sign below 300 nm [30]. The nucleic acid bases tend to

align themselves with their normals parallel to the flow direction.

The difference LD spectra, $\Delta$(LD), of (+)- and (−)-*anti*-BPDE-DNA mixtures, measured within 1–2 min after mixing, and with the DNA contributions subtracted, are shown in fig. 3B and C, respectively. Analogous $\Delta$(LD) spectra for 1-OP-DNA complexes are shown in fig. 4B. These difference LD spectra are defined as:

$$\Delta(\mathrm{LD}) = \mathrm{LD}_{\mathrm{complex}} - \mathrm{LD}_{\mathrm{DNA}} \qquad (6)$$

where $\mathrm{LD}_{\mathrm{DNA}}$ denotes the LD spectrum of the DNA solution measured just before the diol epoxide molecules are added, and $\mathrm{LD}_{\mathrm{complex}}$ that measured just after mixing. The $\Delta$(LD) spectra are negative in sign and resemble in shape the inverted absorption spectra of the noncovalently bound BPDE molecules (fig. 3A, solid line; and fig. 4A). The $\Delta$(LD) signals are less than 5% of the free DNA LD signals at 258 nm, which account for the lower signal-to-noise ratios in the $\Delta$(LD) spectra below 300 nm.

The minima in the LD spectra between 270 and 290 nm, and near 245 nm, suggest that both the *y*- and *z*-polarized transitions of both BPDE stereoisomers and of 1-OP tend to be tilted close to the planes of the DNA bases, as expected for intercalation complexes. In principle, this intercalation model can be confirmed by calculating the ratios $\Delta(\mathrm{LD})/A_{\mathrm{complex}}$ for both the *y*- and *z*-polarized transitions [22]. Such calculations are subject to error for the *y*-polarized absorption band at approx. 280 nm which overlaps the DNA absorption band below 300 nm, because of the uncertainties involved in calculating $A_{\mathrm{complex}}$ in this wavelength region. However, at 354 nm the absorbance is predominantly due to BPDE bound physically to DNA with only a minor contribution from free BPDE molecules. We have thus quantitatively investigated the absorbance and LD properties of the *z*-polarized transition at 354 nm in order to estimate more precisely the angle of orientation of the long axis of the pyrenyl residue. This angle can be estimated from the following ratio [22]:

$$A' = \frac{\mathrm{LD}(354)/A(354)}{\mathrm{LD}(258)/A(258)} \qquad (7)$$

The numerator in this ratio refers to the reduced LD of BPDE complexed to DNA, while the denominator refers to that of the DNA in the absence of BPDE. This ratio is expected to be unity if the transition moments of BPDE are parallel to those of the DNA bases [20,30].

Typical absorption and LD data for noncovalent (+)- and (−)-*anti*-BPDE non-covalent complexes are listed in table 2. In these experiments, a relatively high DNA concentration was utilized (0.74 mM) to ensure relatively high values of $X$ (= 0.90), and thus a high absorbance due to complexed BPDE molecules at 354 nm, and to minimize the already small contribution of free molecules at this wavelength. The absorbances at 354 nm are approximately the same for both enantiomers, as expected, since the values of $K$ are similar for (+)- and (−)-*anti*-BPDE [31].

The calculated values of $A'$ seem to be slightly greater than 1.0, although the experimental uncertainties are such that values of 1.0 cannot be excluded. Values of $A'$ greater than unity could arise from a stiffening and elongation of DNA segments containing intercalated BPDE molecules; these segments could then align slightly better in the flow gradient than those containing no intercalants and whose contributions to the LD at 258 nm are dominant. Elongation of DNA

Table 2

Absorbance ($A$), linear dichroism (LD), and ratios $A'$, of noncovalent BPDE-DNA complexes

[DNA] = 0.76 mM; [BPDE] = 8.6 $\mu$M.

| | $A$ | | LD | | $A'$ |
|---|---|---|---|---|---|
| | 258 nm | 354 nm | 258 nm | 354 nm | |
| (+) enantiomer | 4.9 | 0.12 ± 0.01 | 30.0 | 0.8 ± 0.05 | 1.09 ± 0.11 |
| (−) enantiomer | 4.9 | 0.11 ± 0.01 | 28.8 | 0.75 ± 0.05 | 1.18 ± 0.13 |

upon the formation of ionic drug-DNA intercalation complexes has long been recognized [32].

The fact that $A'$ is close to unity strongly suggests that the mean angle between the $z$ transition moments and the average orientation of the DNA base normals is approx 90°, assuming that these normals are approximately parallel to the axis of the DNA helix [13,20].

The relative amplitudes of the $z$-polarized 354 nm and $y$-polarized 286 nm band are quite similar in the LD and absorption spectra (fig. 3). This suggests that the $y$- and $z$-directions are both parallel to the planes of the DNA bases, as expected for classical intercalation complexes [33–35]. These results are in agreement with previous evidence which indicates that BPDE forms noncovalent intercalation complexes with DNA [16–19, 22–24, 31].

### 3.3. Covalent adducts

#### 3.3.1. (+)-anti-BPDE-DNA

The difference absorption spectrum of a typical adduct derived from the covalent binding of (+)-*anti*-BPDE to DNA is shown in fig. 5A. This spectrum, unlike the absorption spectrum of non-

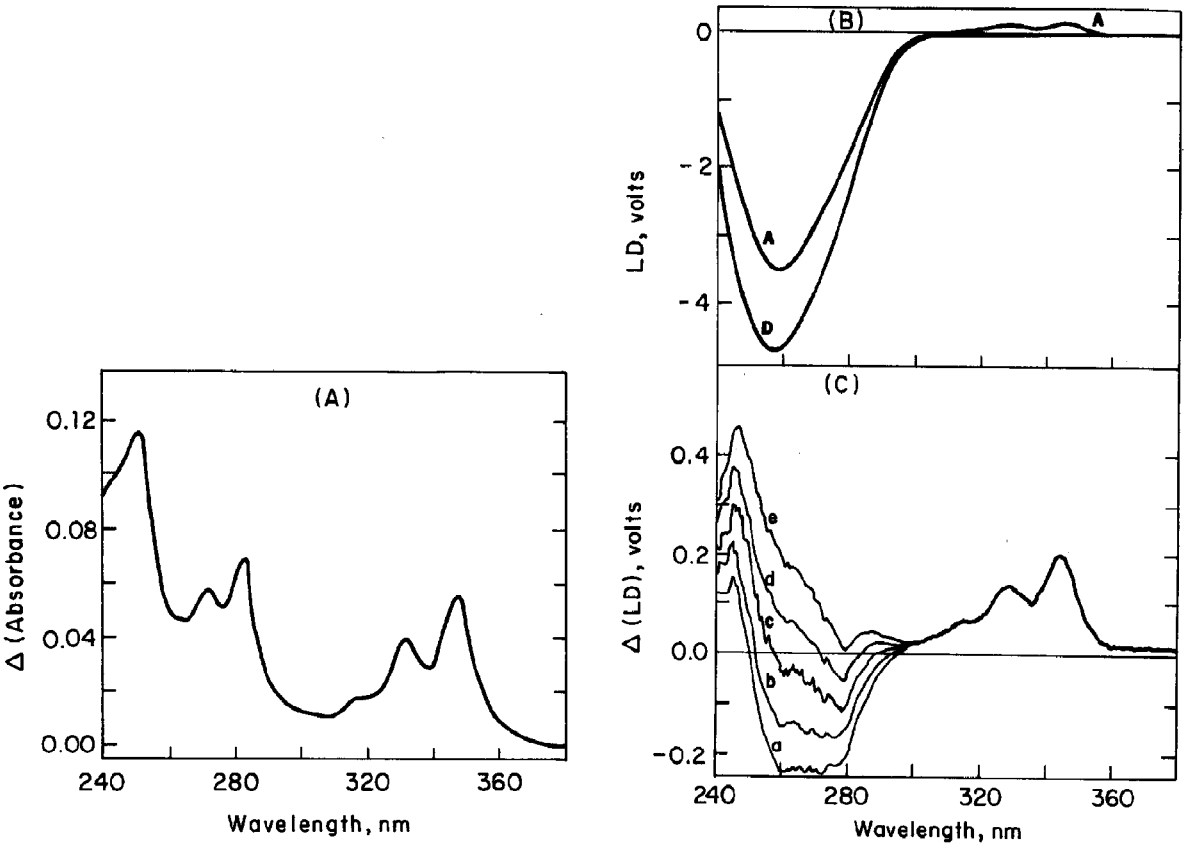


Fig. 5. Difference absorption, LD, and difference LD spectra of covalent (+)-*anti*-BPDE-DNA adducts; [DNA] = 0.15 mM, covalently bound BPDE residues = 1.8 μM. (A) Difference absorption spectrum attributed to covalently bound BPDE residues. (B) LD spectra of DNA before modification (D), and adduct, after covalent modification with BPDE (A). (C) Difference LD spectra determined according to eq. 7 with $p = 0.690$ (a), 0.712 (b), 0.736 (c), 0.759 (d) 0.784 (e). Curve c is normalized at the DNA absorption peak at 258 nm, which corresponds to $p = 0.736$, and is shown with the full contributions of noise to the measured signal; the other curves have been smoothed to reduce the noise.

covalently intercalated BPDE molecules (fig. 3A), exhibits well-defined maxima which are red-shifted by only 2–4 nm relative to those of free molecules in buffer solution (table 1). The relatively small perturbations of the absorption spectrum suggest a relatively low degree of pyrenyl ring-DNA base stacking interactions, and that the pyrene ring system is exposed to the solvent environment [26–29].

LD spectra of the DNA sample before modification, and after the covalent binding of (+)-*anti*-BPDE, are shown in fig. 5B. Above 300 nm, the LD signal is positive in sign and resembles the absorption spectrum; as discussed in detail elsewhere [9], these adducts are of the external site II type [13,21,36,37].

There is a significant overall decrease in the LD signal below 300 nm after the covalent binding of BPDE. This effect was attributed to the formation of bends or kinks at the BPDE-binding sites [38]. However, the decrease in the LD signal can also be attributed to increased local flexibilities of the DNA at or near the binding sites [9,13]; furthermore, the formation of single-strand breaks [39,40], which occur with an efficiency of less than 1%, could also contribute to this effect. This decrease in the intrinsic DNA LD signal indicates that the simple subtraction procedure used in the case of the noncovalent complexes (eq. 6) can no longer be used to obtain the net contribution of the pyrenyl residues to the $\Delta$(LD) spectra. We have established a semiquantitative procedure for minimizing the DNA contributions to the $\Delta$(LD) spectra which is based on the equation:

$$\Delta(\mathrm{LD}) = \mathrm{LD}_{\mathrm{adduct}} - p\mathrm{LD}_{\mathrm{DNA}} \qquad (7)$$

where $\mathrm{LD}_{\mathrm{adduct}}$ and $\mathrm{LD}_{\mathrm{DNA}}$ represent the LD spectrum of BPDE-modified and unmodified DNA, respectively, with $p$ being an adjustable parameter which is less than unity, and which is not known a priori. If $p$ is too large, a large positive contribution of a DNA-like LD spectrum will appear in the difference LD spectrum; on the other hand, if $p$ is too small, a negative DNA-like spectrum will appear in the $\Delta$(LD) spectrum. A series of $\Delta$(LD) spectra corresponding to different values of $p$ are shown in fig. 5C; it is evident that large positive and large negative $\Delta$(LD) spectra are obtained when $p$ is either below 0.69 (curve a), or above 0.784 (e). Normalization at 258 nm corresponds to a value of $p = 0.736$ (curve c), which in turn corresponds to a 36% decrease in the LD signal at 258 nm upon covalent binding of (+)-*anti*-BPDE, in reasonable agreement with recently published data [13].

The normalization of the $\mathrm{LD}_{\mathrm{adduct}}$ and $\mathrm{LD}_{\mathrm{DNA}}$ signals at 258 nm would be rigorously quantitative if there were no contribution of the PAH residue to the LD signal at this wavelength. The contribution of the PAH chromophore near 258 nm may indeed the small because the shape of curve c suggests that the 250 nm band contributes a positive LD signal, while the $y$-polarized band near 280 nm contributes a negative LD signal; thus, the overall contribution of the PAH residue near 258 nm may indeed be very small, since it must be situated near the cross-over point which divides the negative and positive portions of the LD spectrum.

The difference LD spectrum in fig. 5B clearly shows that the LD signal of the $y$-polarized band near 280 nm is negative in sign, while the $^1L_a$ and $^1B_a$ bands are characterized by positive LD signals. Detailed analyses of the LD dichroism characteristics of the $z$-polarized $^1L_a$ band have been published and show that $\theta$ lies in the range of 15–35° [13,21,36,37]. A similar approach for calculating the inclination angle of the $y$-axis is complicated and subject to error because of the uncertainties involved in selecting the correct value of $p$ in the normalization procedure. Nevertheless, Eriksson et al. [13] estimated that the $y$-axis is inclined at an angle of about 70° with respect to the DNA base normals, but the error limits were not stated. However, because of the negative sign of the $\Delta$(LD) signal it can be stated conservatively that the $y$-axis is tilted at angles greater than 55° with respect to the average orientations of the normals to the DNA bases.

Comparisons of the difference LD spectra of the physically intercalated and covalently bound (+)-*anti*-BPDE residues (figs. 3B and 5B show that only the $z$-direction of the pyrene residue undergoes a large change (55–70°) in orientation upon covalent binding, while the orientation of

the $y$ direction does not seem to be altered by more than 20–35°.

*3.3.2. (−)-anti-BPDE-DNA*

The difference absorption spectrum of the covalent adducts, reflecting the net contribution of the pyrenyl residues, is shown in fig. 6A. This absorption spectrum is clearly more diffuse in nature than the spectrum of the covalent (+)-*anti*-BPDE-DNA adducts, and is similar to that of noncovalent complexes (fig. 3A). The $^1B_b$ maximum at 285 nm is also red-shifted by about 7 nm,

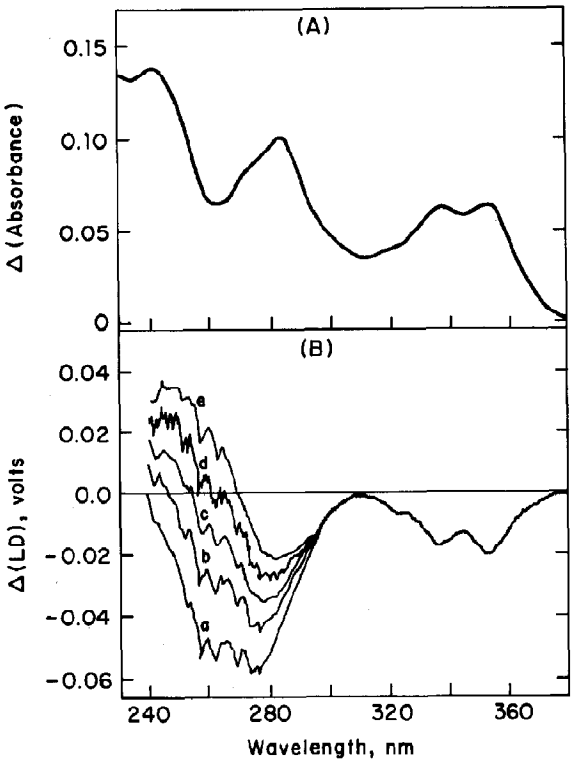


Fig. 6. Difference absorption and difference LD spectra of covalent (−)-*anti*-BPDE-DNA adducts ([DNA] = 0.2 mM; covalently bound BPDE residues, 1.8 μM). (A) Difference absorption spectrum. (B) Difference LD spectra with $p = 0.857$ (a), 0.867 (b), 0.874 (c), 0.880 (d), and 0.886 (e). Curve d represents normalization of the LD spectra of modified and unmodified DNA at 258 nm ($p = 0.88$), and is shown with the full noise contribution. The other curves have been smoothed before recording. The LD signal of the modified sample at 258 nm was −3.7 V.

while the $^1B_a$ transition at 242–244 nm appears to be slightly blue-shifted.

Typical Δ(LD) spectra are shown in fig. 6B. One of these spectra (curve d) represents a normalization of the $LD_{adduct}$ and $LD_{DNA}$ signals at 258 nm ($p = 0.88$). Slightly smaller, or slightly larger values of $p$ produce significant changes in the Δ(LD) spectrum below 300 nm (curves a–c and e, respectively).

Based on selective photodissociation experiments, it has been recently shown that a positive LD signal, resembling in characteristics the site II-type LD spectrum of the (+)-*anti*-BPDE-DNA covalent adducts, is also buried under the overall negative LD spectrum in the $^1L_a$ wavelength region. The (−)-*anti*-BPDE-DNA LD spectrum is thus a superposition of site I and site II adducts, with the quasi-intercalative site I conformations dominating by a factor of 2:1 [26]; as discussed elsewhere [26], these site I conformations could correspond to the wedge-shaped intercalation complexes described by Hogan et al. [38].

The negative LD signal in the 280 nm region is negative in sign for all of the various values of the parameter $p$. This suggests that the $y$-polarized $^1B_b$ transition, like the $z$-polarized transition at longer wavelengths, is also predominantly tilted towards the planes of the DNA bases. Because of the uncertainties involved in the magnitude of the LD signal at 280 nm, the orientation angle $\theta$ of the $y$-axis of the pyrene residue cannot be estimated accurately; nevertheless, the negative LD signal in the 280 nm region, regardless of the value of the parameter $p$ used in the subtraction procedure, indicates that $\theta > 55°$. The angle $\theta$ for the $z$-axis has been reported to be greater than 60° [22,37]. Therefore, overall, the planes of the pyrenyl residues are tilted close to the planes of the DNA bases.

The LD signal within the $^1B_a$ band at 240–250 nm appears to be positive in sign (fig. 6B). This is not an artifact of the subtraction procedure; changing the value of $p$ does not alter the nature of the Δ(LD) signal which tends to increase in the positive direction below 260 nm, irrespective of the value of $p$ selected. Since the $^1B_a$ transition at 240–245 nm is predominantly $z$-polarized [10], it is, at first sight, surprising that the LD signals

corresponding to the $^1B_a$ band at 245 nm and the $^1L_a$ band above 300 nm, are of opposite sign. However, there are several factors which could bring about the differences in signs of the LD signals of these two absorption bands: (1) the $^1L_a$ transition is almost purely polarized along the *z*-direction, while the $^1B_a$ transition is polarized along both the *z*- and *y*-directions, with the *z*-polarization dominating by a factor of about 6 at the $^1B_a$ absorption maximum [12]; (2) the formation of covalent adducts (and noncovalent complexes) gives rise to significant decreases in the molar extinction coefficients of the pyrenyl residues; these decreases may be polarization-dependent, since the interactions which lead to these alterations may be highly anisotropic; (3) the LD signals of the covalent (−)-*anti*-BPDE-DNA adducts are heterogeneous and are superpositions of positive site II and negative site I LD signals [26], with the negative components predominating above 280 nm, as shown in fig. 6B; since the molar extinction coefficients of these two components may change significantly as a function of wavelength, it is possible that the positive LD components dominate the absorption spectrum, and thus the sign of the LD signal, below 260 nm. These factors may account for the apparently anomalous differences in the signs of the LD signals within the $^1L_a$ and $^1B_a$ absorption bands.

The changes in orientation of (−)-*anti*-BPDE upon binding covalently to DNA are quite different from those of the (+) enantiomer. Evidently, the intercalation geometry in the noncovalent complexes places the epoxide rings of the two enantiomers in different positions for reaction with the DNA bases [33–35]. Both the *z*- and *y*-axes of a dominant fraction of the (−) enantiomer appear to undergo little change in orientation as a result of the covalent binding reaction, giving rise to adducts with site I type conformations.

### 3.3.3. *1-OP-DNA adducts*

The characteristics of the covalent adducts derived from the binding of 1-OP to DNA, even though the LD signals have the same sign within the $^1L_a$ band above 310 nm (fig. 7), are quite different from those of the (−)-*anti*-BPDE-DNA noncovalent complex (fig. 4). While in the latter

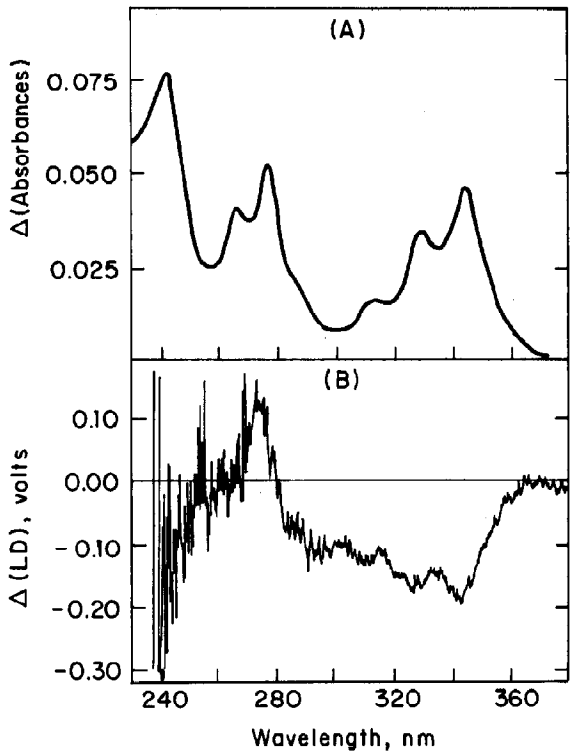


Fig. 7. Difference absorption and difference LD spectra of covalent 1-OP-DNA adducts ([DNA] = 0.17 mM, covalently bound 1-OP residues = 1.6 μM). (A) Difference absorption spectrum (contribution of covalently bound 1-OP residues). (B) Difference LD spectrum (normalization of unmodified DNA and modified DNA adduct spectra at 258 nm).

case the red shift in the $^1L_a$ absorption band is about 10 nm, in the 1-OP adducts this shift is only 3 nm. The $^1B_b$ absorption maxima are also shifted by about 3 nm, while the $^1B_a$ maximum at 241–242 nm remains unshifted, as in the case of the (+)-*anti*-BPDE-DNA covalent adducts.

In the noncovalent complex the Δ(LD) signal is negative over the entire wavelength range as expected for an intercalative conformation. In the covalent adducts, the LD signals of the $^1L_a$ and $^1B_a$ transitions are still negative in sign, but the LD signal corresponding to the *y*-polarized $^1B_b$ transition is positive in sign. It is evident that upon covalent binding, the *z*-direction of the pyrenyl moiety of 1-OP does not change its orientation relative to the planes of the DNA normals,

while the $y$-direction becomes tilted closer to these normals.

The mean orientation angle of the $z$-direction calculated according to eq. 6 is $65 \pm 3°$ [17], while the positive sign of the LD signal around 275 nm indicates that the orientation angle of the $y$-direction is less than 55°. Based on these data, it is concluded that the covalently bound pyrenyl residues in the 1-OP-DNA adducts are not intercalated. The rather sharp absorption spectra of the pyrenyl residues and the small red shifts also suggest that pyrene-DNA base stacking interactions are considerably less significant than in the case of the covalent (−)-*anti*-BPDE-DNA adducts. The observations that the pyrenyl residues in the covalent 1-OP-DNA adducts are exposed to the solvent environment [27] are also in agreement with these conclusions.

## 4. Conclusions

The LD methods described here are capable of revealing the orientations of both the long ($z$) and short ($y$) axes of the pyrene residues of 1-OP and BPDE relative to those of the DNA bases, even though the absorption band due to the short-axis transition moment overlaps with the DNA absorption band below 300 nm. The determination of LD spectra of the aromatic chromophores below 300 nm is complicated by the fact that the intrinsic LD of the DNA molecules is reduced by the introduction of flexible joints or kinks at the covalent carcinogen-DNA binding sites. However, the contributions of the PAH chromophores to the LD spectra, and thus the orientations of the ultraviolet (240–300 nm) transition moments can be studied by utilizing a parametrized subtraction procedure described here.

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