Binding Geometry of Benzo[*a*]pyrene Diol Epoxide Covalently Bound to DNA. A Flow Linear Dichroism Study

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Flow linear dichroism (LD) provides new structural information on 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-BPDE) covalently bound to DNA: by resolving the LD spectrum with respect to the polarized absorption components of the pyrenyl chromophore, we have determined the orientations of the in-plane symmetry axes of the bound compound relative to the helix axis of DNA [they are on average oriented at 70° (short axis) and 30° (long axis)]; for both (+)- and (−)-*anti*-BPDE adducts the flexibility of the DNA is increased.

The variation in carcinogenic potency between the different stereoisomers of 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) makes studies of the nature of the DNA interaction with these compounds highly interesting. All BPDE isomers bind covalently to the bases of DNA, with a base specificity that varies between the different stereoisomers.[1-4] Of the four possible BPDE isomers [(+)- or (−)-*syn* and (+)- or (−)-*anti*], the (+)-*anti* enantiomer is by far the most carcinogenic form.[5,6] The observation of a unique binding geometry of this enantiomer in its complex with DNA is therefore a spectacular finding, possibly of mechanistic significance: in contrast to a dominating intercalative binding mode of the other isomers, (+)-*anti*-BPDE forms a complex with DNA in which the pyrenyl chromophore is more parallel than perpendicular to the helix axis.[7-9] According to their spectroscopic features the BPDE–DNA complexes can be divided into two classes, the 'type I' complex being characterized by a negative linear dichroism (LD) and a 10 nm

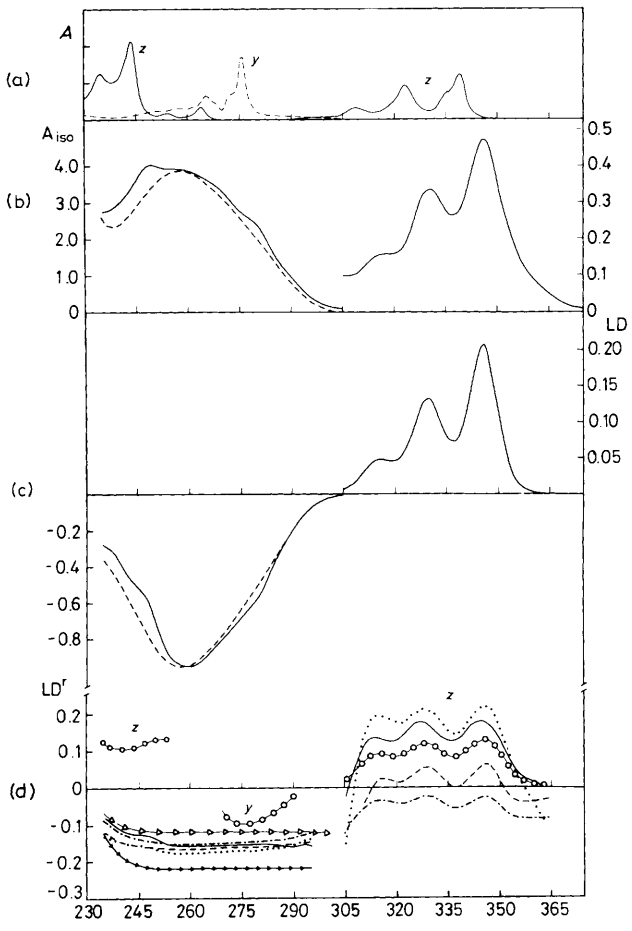


Figure 1. (a) Polarized component spectra of the pyrenyl chromophore:[13,16] solid and broken curves refer to *z*- and *y*-polarized absorption spectra, respectively. (b) Isotropic absorbance spectrum of (±)-*anti*-BPDE–DNA complex (0.59 mM phosphate, binding ratio r = 0.025, *i.e.* one BPDE per 20 base pairs). Broken curve: pure DNA. (c) Linear dichroism spectrum of (±)-*anti*-BPDE–DNA complex (r = 0.025). Flow gradient: 3040 s^{-1}. (d) Reduced linear dichroism spectra ($LD^r = LD/A_{iso}$) of (±)-*anti*-BPDE–DNA complex (r = 0.025). Resolved LD^r components: pyrenyl chromophore (○), DNA chromophore (▷). LD^r spectra of (+)-*anti*-BPDE–DNA (· · ·) (r = 0.0067), (±)-*anti*-BPDE–DNA (—) (r = 0.0096), (−)-*anti*-BPDE–DNA (- - -) (r = 0.0096), and (±)-*syn*-BPDE–DNA (-·-) (r = 0.0060). LD^r spectrum of unmodified DNA (▶).

red-shift ot the 300—350 nm absorption bands, and also a considerable fluorescence quenching, whereas the 'type II' complex exhibits a positive LD and only a 2—3 nm red-shift of the absorption bands.[7—9] DNA, in particular the exocyclic amino group of guanine, reacts selectively with the (+)-enantiomer of racemic *anti*-BPDE (≥90%)[10] forming a type II complex, while the (−)-enantiomer results in about equal amounts of each type. While intercalation-like binding seems to be an essential feature of the type I complexes, the structural characteristics of the type II complexes have not been unambiguously determined. The positive LD excludes intercalation in the classical sense and Geacintov *et al.* have proposed an external binding where the pyrenyl group resides in the minor groove of DNA.[11] Hogan, on the other hand, has suggested a quasi-intercalative 'wedge-like' complex, where the BPDE causes a bend in the DNA helix.[12]

Here we report results that will further aid in elucidating the three-dimensional structure of the *anti*-BPDE–DNA complexes. Figure 1 depicts flow linear dichroism spectra of DNA modified by different BPDE isomers together with the resolved absorption components of the pyrenyl chromophore (for an introduction to the LD method for studying DNA conformation and interactions, see refs. 13–15). The reduced linear dichroism, LD^r, defined at any wavelength as the ratio $LD(\lambda)/A_{iso}(\lambda)$, where A_{iso} is the absorbance of the isotropic sample, can be related to the orientations of the transition moments responsible for the light absorption according to equation (1).[14]

$$LD^r(\lambda) = \frac{\Sigma F_i \varepsilon_i(\lambda)\,(LD^r)_i}{\Sigma F_i \varepsilon_i(\lambda)} \qquad (1a)$$

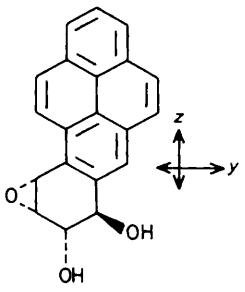
$$(LD^r)_i = \frac{3}{2} S \cdot (3 < \cos^2\alpha_i > - 1) \qquad (1b)$$

The summation in (1a) is over all contributing absorption bands and chromophoric groups [F_i being the fractional concentration of each component and $\varepsilon_i(\lambda)$ the extinction coefficient]. In equation (1b) S is an orientation factor ($0 \leqslant S \leqslant 1$) for the DNA helix and α_i the angle between the respective transition moment and the helix axis. For B form DNA an 'apparent' α_{DNA} at 260 nm equal to 86° can be used.[14] The same effective base orientation will be assumed also for the modified DNA as will be mentioned below. The orientation factor, S, was determined according to equation (1) from LD^r at 260 nm where only a small correction for the pyrene absorption had to be made.

After subtraction of the DNA contributions, an $LD^r(\lambda)$ spectrum of bound BPDE is obtained as shown in Figure 1d for *anti*-BPDE at a BPDE : DNA phosphate concentration ratio r = 0.025. As seen from the resolved polarized spectrum of pyrene, the absorptions at 275 nm and 346 nm are purely *y*- and *z*-polarized, respectively. LD^r at these wavelengths, together with the determined S, directly give $<\cos^2\alpha_i>$ values, according to equation (1). These can be interpreted in terms of apparent angles of the *y* and *z* axes of the pyrenyl chromophore relative to the helix axis: α_y = 70° and α_z = 30°.

The *anti*-BPDE binding site (the exocyclic amino group of guanine) is located in the minor groove of DNA. Inspection of molecular models indicates that the binding angles are not compatible with a location of the adduct in an undisturbed minor groove. This, in turn, suggests a local structural disturbance of the helix at the binding site. Since the 320—360 nm region of pyrene is almost purely *z*-polarized, LD^r for a single type of BPDE–DNA complex is expected to be constant. As seen in Figure 1d, LD^r is not in general constant but shows significant maxima and minima. As can be seen from equation (1a) a varying $LD^r(\lambda)$ from a purely polarised adsorption band must derive from several contributions with different $(LD^r)_i$ values and $\varepsilon_i(\lambda)$ components that are shifted differently in wavelength. The present results therefore imply the existence of more than one binding environment with different red-shifts and different binding angles. An orientational variation can derive from identically bound adducts distributed over a continuous set of orientations or from the presence of different discrete binding sites. Qualitatively similar variations are seen for all of the isomers in Figure 1d. The LD^r variations seem insensitive to the r-value, suggesting an orientational distribution, possibly dynamic, in an inhomogeneous environment. A more detailed analysis of the corresponding angular distributions is in progress.

The considerably diminished LD^r amplitude of the DNA



(+)-*anti*-BPDE

absorption, observed upon BPDE modification of DNA, can be due to an impaired DNA orientation, S in equation (1b), or to a changed local average conformation of the DNA bases (the 'optical' $<\cos^2\alpha>$ factor). There is strong evidence that the optical factor is essentially unchanged: (i) a retained shape of the LD contribution from the DNA bases shows that these are not preferentially tilted, (ii) the ratio between the LD^r values of the pyrene (346 nm) and the DNA (258 nm) absorptions, when plotted *vs.* r, is constant (results not shown). Thus, the effect of *anti*-BPDE-modification is a decreased S (by some 50% for $r = 0.025$), most likely due to a considerably increased flexibility of the DNA helix. Preliminary results indicate a higher relative perturbation of the orientation (a lowered S) at low degrees of modification. This is also expected if the rigid structure of DNA is interrupted by the occurrence of 'flexible joints' at the sites of *anti*-BPDE modification.

In conclusion, the LD results show that *anti*-BPDE not only violates the local DNA structure (*anti*-BPDE can neither be intercalated nor accommodated in an unperturbed minor groove) but also introduces a considerable flexibility into the DNA helix.

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