Biochemical Pharmacology, Vol. 37, No. 9, pp. 1859–1860, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

STRUCTURAL PROPERTIES OF THE COVALENT (+)-*anti*-BPDE-poly(dG-dC)(dG-dC) COMPLEX

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Introduction

The common environmental pollutant benzo-(a)pyrene diol epoxide (BPDE) is a polyaromatic hydrocarbon wellknown for its mutagenic potency. There are four isomers of BPDE, of which 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo-(a)pyrene ((+)-*anti*-BPDE) is by far the most carcinogenic. Upon covalent binding to DNA, the (+)-*anti*-isomer has a great specificity for the exocyclic aminogroup of guanine (N-2, ref. 1). The (+)-*anti*-BPDE-DNA complex is characterized by spectroscopic properties which differ considerably from the other BPDE-DNA complexes: a small (~3nm) red shift in light absorption, a positive linear dichroism (LD) and only modest quenching of the fluorescence (ref. 2). It may be relevant to correlate the biological activity of (+)-*anti*-BPDE to the geometrical nature of its complex with DNA: The long axis of the pyrene chromophore is nearly parallel to the DNA helix axis (~30°) while the short axis is more perpendicularly oriented (~70°) (ref. 3). The most probable location of the BPDE chromophore is in the minor groove of DNA. For a smaller part of the complex the binding geometry has similarities with intercalation, judging from a larger red shift (~12 nm), a negative LD and strong fluorescence quenching (ref. 2). The reduced linear dichroism (LD divided by the isotropic absorbance) of the (+)-*anti*-BPDE bound to DNA varies markedly with wavelength (ref. 4) which evidences a heterogeneous orientational distribution of the bound chromophores.

In order to characterize the complex described we have chosen to study the (+)-*anti*-BPDE poly(dG-dC) complex in which one chemical species should be completely dominating. Attempts are made to mimic the features of the LD^r spectrum by assuming a monotonic dependence of the absorption red shift on the BPDE binding angle relative to the DNA helix axis. From LD^r we will conclude a rather wide orientational distribution which by fluorescence methods will be shown to be dynamic.

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Methods

(+)-*anti*-BPDE (>95% pure) and double stranded alternating poly(dG-dC) (P&L Biochemicals) was used. The samples were kept in 10 mM NaCl, 10 mM sodium cacodylate adjusted to pH 7.0. The procedure of modification is given in detail in ref. 5. Linear dichroism was measured differentially using polarization modulation in a modified circular dichrometer (Jasco J-500) with flow orientation obtained in a Couette cell (as described in ref. 6). Isotropic absorption was measured on a Cary 219 spectrophotometer and fluorescence on an Aminco SPF-500 spectrofluorometer.

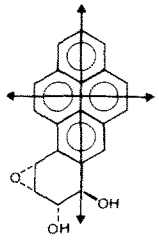
Linear dichroism is defined as the difference in absorption between two orthogonal polarizations, $LD=A_{\parallel}-A_{\perp}$, $\parallel$ denoting parallel to the flow direction. The reduced linear dichroism is measured as: $LD^r(\lambda)=LD(\lambda)/A_{iso}(\lambda)$ and can be split in contributions from the different absorbing bands and their orientations:

$$LD^r = \sum_i Q_i \epsilon_i(\lambda)(LD^r)_i \;/\; \sum_i Q_i \epsilon_i(\lambda)$$

$$(LD^r)_i = 3/2\ S\ (3\langle\cos^2\alpha_i\rangle - 1)$$

where Q_i and $\epsilon_i(\lambda)$ means the fractional contribution and the extinction coefficient of component i at wavelength λ, respectively. α_i denotes the angle between the transition moment responsible for the absorption band i and the polynucleotide helix axis. S is an orientation parameter ($0 \leq S \leq 1$) equal to 1 for perfect orientation. Fluorescence polarization anisotropy, $FPA=(I_{\parallel}-I_{\perp})/I_{\parallel}+2I_{\perp})$, $I_{\parallel}$ and $I_{\perp}$ being the intensities of the steady state fluorescence polarized parallel and perpendicular to the polarized exciting light.

In the simulations performed the distribution of BPDE chromophores is varied over the angular range 0°–90° The population of BPDE molecules is divided into fractions characterized by their angle, α_i, to the helix axis. The spectral shifts were modelled by $\Delta\lambda_i = \Delta\lambda^{solvent} + \Delta\lambda^{\circ}\sin\alpha$ being zero for pure pyrene in ethanol (the spectrum used as a basis in the simulations). Here $\Delta\lambda^{solvent}$ and $\Delta\lambda^{\circ}$ are set to 3 and 12.5 nm respectively, as concluded experimentally. Each fraction is given a value $LD_i^r = \text{const}(3\cos^2\alpha_i - 1)$ where the constant is chosen to compare with the experiments.



(+)-*anti*-BPDE

Results and Discussion

The LD^r spectrum of (+)-*anti*-BPDE-poly-(dG-dC) (Fig. 1) is positive and shows a pronounced wavelength dependence in the BPDE region (300-350 nm) while it is negative in the nucleotide region (< 300 nm) and shows no great variation with wavelength. The constant LD^r in the nucleic absorption region may be taken as evidence that the average DNA conformation is not significantly changed. The wavelength dependence in LD^r indicates that the BPDE chromophores are distributed over a wide angular range. This can be understood by realizing that the absorption spectrum is shifted differently for different degrees of interactions with the bases. The corresponding LD will in turn get different contributions depending on the angle between the BPDE chromophore and the orientation axis.

In the simulation of the LD^r spectra a number of distribution models were tried: I. A Gaussian distribution around one angle to be considered as the most favourable conformation, II. An unequal distribution between two discrete angles (delta functions), III. Two unequally populated favoured conformations, represented by two Gaussian distribution functions and IV. a wide angular domain isotropically populated. In the Gaussian distribution also the standard deviations were varied. In Fig. 2 the simulated spectra of the models I to IV (those giving the best agreement with the experimental result) are shown. By means of the average LD^r amplitude and the amplitude of the spectral wavelike structure the goodness of the simulation can be measured. It is concluded that two Gaussian distributions, one at 20° and one at 70° populated to 80% and 20%, respectively, give the best description of the (+)-*anti*-BPDE-poly(dG-dC) complex.

Fluorescence measurements of the pyrene emission show almost complete depolarization within the fluorescence lifetime (~3.7 ns, ref. 7). This implies rapid mobility of the chromophore when bound to poly(dG-dC). Probably an interchange occurs between the two preferred orientational states of the pyrene long axis around 20° and 70°. An inhomogeneous BPDE environment is also evidenced by different emission profiles when exciting at different wavelengths. Another striking difference is that excitation at 355 nm gives a significantly stronger fluorescence from pyrene excimers, compared to excitation at 340 nm. The excimer formation of BPDE bound to DNA only occurs within very short BPDE-BPDE distances (3.4 Å, ref. 7) which should be difficult to attain without distortion of the poly(dG-dC) conformation. This is also in agreement with a pronounced increase in DNA flexibility, seen as lowered LD^r amplitude in the DNA band, when increasing the *anti*-BPDE/DNA ratio.

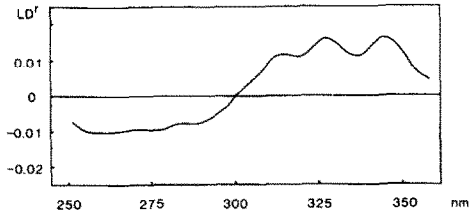


Figure 1. Reduced linear dichroism of (+)-*anti*-BPDE- poly(dG-dC) 0.30 mM phosphate, at binding ratio 0.064.

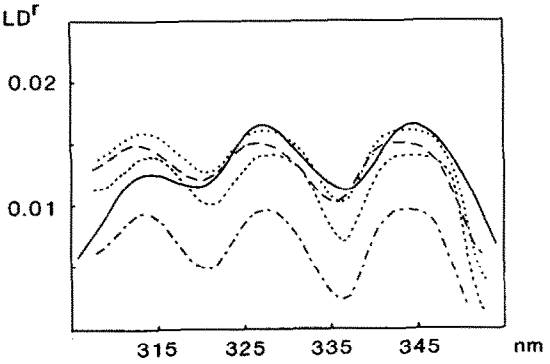


Figure 2. Simulated LD^r spectra: (– – –) single Gaussian, (— —) two states, (•••) double Gaussian, (—•—)limited isotropic domain, (——) experimental.

Summary

The structure of the covalent (+)-*anti*-BPDE-poly(dG-dC) complex can be represented by two preferred orientations of the pyrene moiety; one at about 20° relative to the helix axis and one at about 70°, populated as 4:1. A rapid mobility of the BPDE may allow an exchange between the two orientations. The poly(dG-dC) structure becomes more flexible by (+)-*anti*-BPDE modification, seen as a shortened persistence length. This complex may be significant as a model for DNA interaction with covalently binding polyaromatic carcinogens.

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