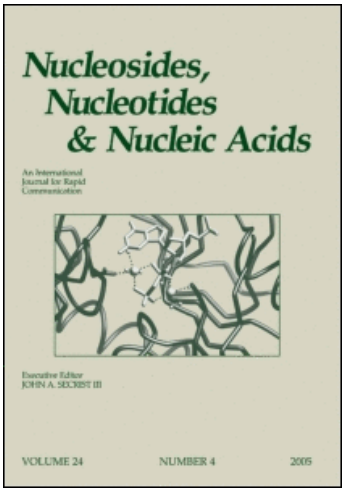

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Structure of a Covalent Complex Between Benzo(a)Pyrene Diol Epoxide and DNA

Magdalena Eriksson[a]; Bengt Nordeh[a]; Bengt Jernström[a]
[a] Dept. of Physical Chemistry, Chalmers University of Technology, Göteborg, Sweden

To cite this Article Eriksson, Magdalena , Nordeh, Bengt and Jernström, Bengt(1988) 'Structure of a Covalent Complex Between Benzo(a)Pyrene Diol Epoxide and DNA', Nucleosides, Nucleotides and Nucleic Acids, 7: 5, 717 — 720
To link to this Article: DOI: 10.1080/07328318808056316
URL: http://dx.doi.org/10.1080/07328318808056316

NUCLEOSIDES & NUCLEOTIDES, 7(5&6), 717-720 (1988)

STRUCTURE OF A COVALENT COMPLEX BETWEEN BENZO(*a*)PYRENE DIOL EPOXIDE AND DNA

Magdalena Eriksson, Bengt Nordén and Bengt Jernström
Dept. of Physical Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden.

Abstract: Flow linear dichroism and fluorescence spectroscopy show that the covalent (+)-*anti*-BPDE-DNA complex adopts two rapidly interchanging conformations. The binding introduces local flexibility in DNA facilitating further covalent attacks.

Introduction

The environmental pollutant benzo(*a*)pyrene can be metabolically converted to benzo(*a*)pyrene diol epoxides of which 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene ((+)-*anti*-BPDE, Figure 1) is wellknown as a strong carcinogen.[1] (+)-*anti*-BPDE forms covalent bonds DNA, highly specifically by trans attack to the exocyclic aminogroup of guanin (N-2)[2], located in the minor groove of DNA. The structure of the (+)-*anti*-BPDE-DNA adducts has been described in terms of two types. Type I is of intercalation-like geometry with the pyrene chromophore more or less parallel to the DNA bases, as seen from spectroscopic studies; ~10 nm red shift and negative linear dichroism (LD) of the 300-350 nm pyrenyl absorption band, and strong fluorescence quenching. Type II exhibits a smaller red shift (2-3 nm) in the absorbance and positive LD,[3] therefrom it has been concluded that the pyrene long axis is close to parallel with the DNA helix axis and it is thought that the BPDE resides in the minor groove.[3] The (+)-*anti*-isomer of BPDE predominantly (>90%) forms type II adducts.[4] Prior to covalent bond formation the BPDE intercalates physically between DNA base pairs from where the covalent binding sites are slowly found (within ~30 minutes).[5]

The aim of later years studies has been to characterize the detailed structure of the two types of adducts. Here we will concentrate on the (+)-*anti*-BPDE-DNA adduct (type II) and show from polarized light and fluorescence spectroscopy that conclusions can be made regarding binding geometry, effects on DNA structure and cooperativity in the binding process.

Materials and methods

Materials. For covalent modification (+)-*anti*-BPDE (>95% pure) and calf thymus DNA (Sigma, type I) or poly(dG-dC) (P&L Biochemicals) were used (see ref 6 for details). The samples were kept in 10 mM NaCl, 10 mM sodium cacodylate at pH 7.0. Isotropic absorption spectra were recorded on

a Cary 219 spectrophotometer, fluorescence on an Aminco SPF–500 spectrofluorimeter and linear dichroism on a Jasco J–500 circular dichrometer, as described elsewhere.[6]

Methods. Linear dichroism (LD) is defined as the difference in polarized absorption between two orthogonally measured directions: $LD=A_{\parallel}-A_{\perp}$. LD can be normalized to the reduced linear dichroism, $LD^r(\lambda) = LD(\lambda) / A_{iso}(\lambda)$ and split into contributions from the different absorbing components, Q_i, and their respective orientation angles relative to the orientation axis, a_i:

$$LD^r = \sum_i Q_i \epsilon_i(\lambda) (LD^r)_i / \sum_i Q_i \epsilon_i(\lambda) \quad 1a$$

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

S is an orientational parameter ($0 \leq S \leq 1$), equal to 1 for perfect alignment. Fluorescence polarization anisotropy $FPA = I_{\parallel} - I_{\perp} / (I_{\parallel} + 2 I_{\perp})$.

Results and discussion

Binding geometries. The geometry of the (+)–*anti*–BPDE–DNA adduct can be characterized from LD measurements. In Figure 1 it is seen that the contribution from BPDE to the LD signal is positive in the 300–350 mn and 230–250 nm absorption bands and negative between 270 and 290 nm. By use of equation 1, approximate angles of the BPDE transition moments relative to the average helix axis can be calculated. The positive LD (corresponding to the pyrene z–axis) gives an angle $\alpha_z \sim 30^0$ while the negative LD (y–axis)gives $\alpha_y \sim 70^0$. The pronounced variation in LD^r with wavelength seen at 300–350 nm indicates an inhomogeneity of the BPDE orientation. To characterize this inhomogeneity, a number of orientational distributions were assumed and corresponding LD^r–spectra were simulated (see ref 6) and compared to experimental results. For comparison, (+)–*anti*–BPDE–poly(dG–dC) was considered, due to its high predominance of type II adducts. It was found that two widely separated angular domains, one around 20^0 and one around 70^0, were needed to account for the experimentally observed peak and trough features. To reproduce the average LD^r amplitude a larger fraction (~80%) must occupy the low–angle domain. The angular distributions within the domains are quite well described by Gaussian functions with standard deviations of 10^0. (Accounted for in detail in ref. 6.)

Fluorescence polarization experiments give small values of the anisotropy, $FPA < 0.05$, when exciting in either the y or z polarized absorption bands. This indicates considerable reorientation of the pyrene chromophore during the fluorescence lifetime. Though longer lifetimes have been observed (for which DNA twisting motion can not be neglected as a source of depolarization), about 50% of the fluorescence intensity comes from a species with τ_F=3.7 ns,[7] which, if substantially immobilized, would have given rise to a larger value of FPA.

In fluorescence emission spectra (Figure 2) the appearance of an excimer band growing with degree of modification, evidences close contacts between BPDE chromophores when bound to poly(dG–dC). In pyrene excimers the molecular distance has been estimated to 3.4 Å.[8] In calf thymus DNA, where the probability of neighbouring GC–basepairs is smaller, the excimer intensity is consequently much lower. In order to adopt a geometry allowing excimer formation, a flexible attachment of the BPDE molecules must be required.

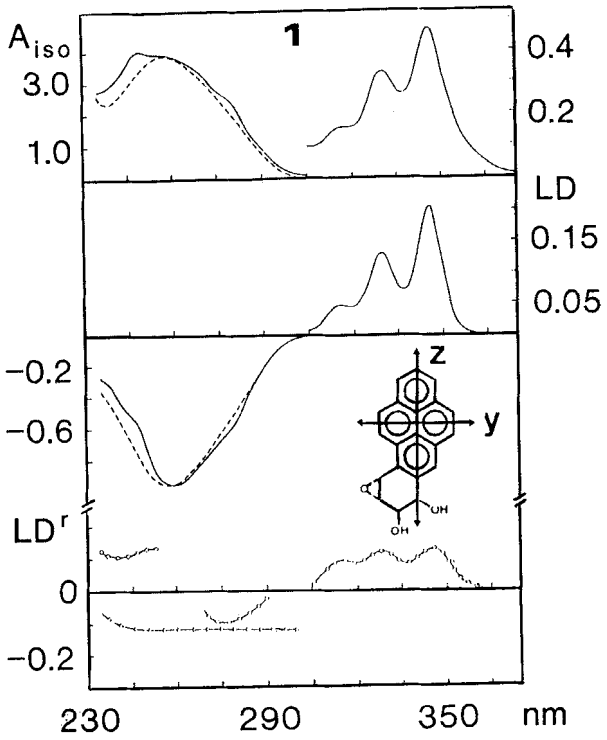

Figure 1. A_{iso}, LD and LD^r spectra of (+)–*anti*–BPDE–DNA (——),.DNA contribution (– – –). 0.025 BPDE/nucleotide, 0.59 mM nucleotide.

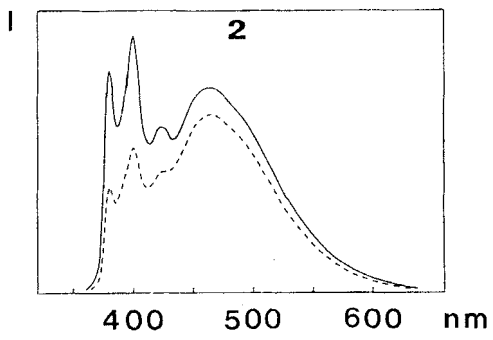

Figure 2. Fluorescence spectra of (+)–*anti*–BPDE–poly(dG–dC), λ_{ex}=350 nm (——),λ_{ex}= 355 nm (– – –). 0.02 BPDE/nucleotide, 45 μM nucleotide.

Different fluorescence emission profiles when exciting at different wavelengths indicate the presence of pyrene chromophores in different environments, as was also concluded from the LD results.

DNA flexibility. When increasing the degree of BPDE–modification of DNA, the LD^r amplitude of DNA decreases by some 50% at a (+)–*anti*–BPDE/DNA–base ratio of 0.03. A similar decrease is seen in the LD^r amplitude of the BPDE chromophore, indicating that the DNA becomes less welloriented (S in equation 1b decreases). This may be due either to introduction of stiff bends or to increased flexibility at the BPDE binding sites. Considering the adduct flexibility required for excimer formation, a dynamic DNA flexibility is the more attractive model.

BPDE–Binding process. Our studies of excimer and monomer fluorescence at various BPDE/poly(dG–dC) ratios, indicate cooperativity in the covalent (+)–*anti*–BPDE–dG bond formation. The relative concentration of excimers $[c_{excimers}/c_{BPDE,total}]$ increases drastically at low degrees of modification and seems to approach a constant value at modifications higher than 5%. This demonstrates facilitation of binding at positions close to already modified guanines, possibly due to local alteration or flexibility in the DNA structure.

REFERENCES

1. Conney, A. H. (1982) *Cancer Res. 42*, 4875–4917.
2. Koreeda, M., Moore, P. D., Wislocki, P. G., Levin, W., Conney, A. H., Yagi, H. & Jerina, D. M. (1978) *Science 199*, 778–781.
3. Undeman, O., Lycksell, P.–O., Gräslund, A., Astlind, T., Ehrenberg, A., Jernström, B., Tjerneld, F. & Nordén, B. (1983) *Cancer Res. 43*, 1851–1860.
4. Meehan T. & Straub, K. (1979) *Nature 277*, 410–412.
5. Geacintov, N.E., Yoshida, H. K., Ibanez, V., Jacobs, S. & Harvey, R. G. (1984) *Biophys. Biochem. Res. Commun. 122*, 33–39.
6. Eriksson, M., Nordén, B., Jernström, B. & Gräslund, A. (1988) *Biochemistry 27*, 1213–1221.
7. Jernström, B., Lycksell, P.–O., Gräslund, A. & Nordén, B. (1984) *Carcinogenisis 5*, 1129–1135.
8. Birks, J. B. (1980) *Photophysics of Aromatic Molecules*, Wiley, New York.