Observation of Excimer Formation in the Covalent Adducts of 9,10-Epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8-diol with poly(dG-dC)

Magdalena Eriksson,[a] Bengt Nordén,[a]* Bengt Jernström,[b] Astrid Gräslund,[c] and Per-Olof Lycksell[c]

[a] *Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden*
[b] *Department of Toxicology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden*
[c] *Department of Biophysics, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden*

The covalent binding of 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8-diol to alternating poly(dG–dC) results in extensive occurrence of excimers as shown by fluorescence spectroscopy; the origin is inferred to be close-lying pyrene chromophores bound to guanines in the minor groove of DNA.

As a result of the biological activity of the different isomers of 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8-diol (BPDE), their covalent interactions with DNA have been studied extensively, mainly by means of optical techniques.[1,2] Of four isomers, (+)-*anti*-BPDE is known to be by far the most carcinogenic.[3] Complexed with DNA, (+)-*anti*-BPDE exhibits spectroscopic properties clearly distinguished from those of the other isomers.[1,2,4] The long axis polarized transition moment of the pyrene chromophore is found to be oriented nearly parallel to the helix axis of the polynucleotide, whose flexibility is remarkably increased by (+)-*anti*-BPDE-modification.[5]

However, the structural details of the (+)-*anti*-BPDE–DNA complex are not yet completely understood. The discovery of pyrenyl excimer formation of (+)-*anti*-BPDE in poly(dG–dC), reported here, has structural implications as to the possible steric arrangements of the covalently attached BPDE, and may be useful for spectroscopic studies of the BPDE–DNA complexes.

Figure 1(a) and (b) show emission spectra of (+)-*anti*-BPDE covalently bound to poly(dG–dC) at different excitation wavelengths and binding ratios. A variation of spectral shape with the excitation wavelength indicates that pyrene chromophores are present in different environments. The broad structureless emission band observed above 400 nm is due to pyrene excimers. Its features correlate well with the classical excimer spectrum observed in concentrated pyrene solutions.[6] The extent of excimer formation in BPDE–poly(dG–dC) is insensitive to dilution of the sample (which excludes the possibility that the excimers are due to association of free BPDE), but increases markedly with increasing degree of modification of the polynucleotide. The excimers are observed also in shorter poly(dG–dC) fragments, such as sonicated poly(dG–dC), and even in the $(dG–dC)_6$ dodecamer, where intramolecular nucleotide–nucleotide contacts due to bending are eliminated. This demonstrates that the excimer is formed between BPDE moieties bound to the same DNA helix.

(+)-*anti*-BPDE binds highly selectively to the exocyclic amino-group (N-2) of guanine,[7] facing the minor groove of DNA. Therefore the excimers should also be located in the minor groove. The fluorescence quantum yield of the monomer is more sensitive to O_2 quenching than is the excimer, suggesting that the structure of the monomer adduct is more accessible to solvent. The accessibility to quenching of (+)-*anti*-BPDE is consistent with a binding of this isomer in one of the grooves, with a distorted structure of the DNA backbone at the binding site, and an increased DNA flexibility, as shown by flow linear dichroism.[5,8] In addition, a pronounced ionic strength dependence is observed, with more excimers at high NaCl concentration. This suggests that excimer formation is favoured by DNA flexibility at the binding positions, facilitating close BPDE–BPDE contacts as the phosphate–phosphate repulsions are reduced. The excimer geometry, with a very short pyrene–pyrene distance (3.4 Å),[6] implies that the two interacting BPDE chromophores are bound to adjacently positioned guanines in poly(dG–dC). Finally, it may be noted that in calf thymus

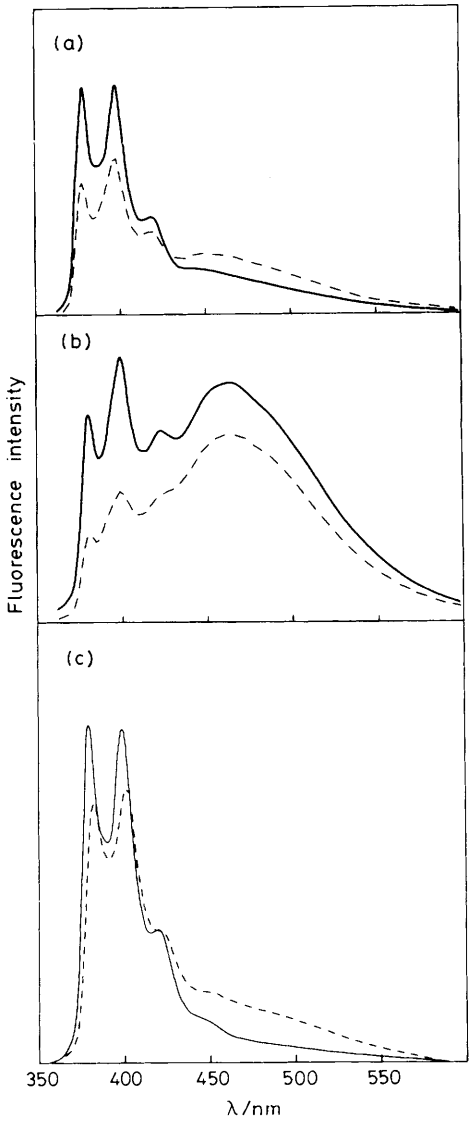

Figure 1. Fluorescence emission spectra of (+)-*anti*-BPDE–poly(dG–dC) (45 μM phosphate, 10 mM NaCl) at binding ratios (a) 0.005 BPDE/phosphate, (b) 0.02 BPDE/phosphate, and (c) (+)-*anti*-BPDE–DNA (0.33 mM phosphate), 0.03 BPDE/phosphate. Excitation wavelengths in (a) and (b): 350 nm (——) and 355 (– – –); in (c): 340 nm (——) and 355 nm (– – –). Vertical axis: fluorescence intensity (arbitrary scale).

DNA [see Figure 1(c)], where the frequency of neighbouring guanines is small compared to poly(dG–dC), excimer fluorescence is consequently very low.

This study was supported by grants from The Swedish Natural Science Research Council and Magn. Bergwalls Stiftelse. We also gratefully acknowledge support from The Swedish Work Environmental Fund and The Swedish Cancer Society. We thank Dr. Kjell Sandros for helpful discussions.

Received, 15th June 1987; Com. 838

References

1 N. E. Geacintov, A. G. Gagliano, V. Ibanez, and R. G. Harvey, *Carcinogenesis*, 1982, **3**, 247.
2 O. Undeman, P.-O. Lycksell, A. Gräslund, T. Astlind, A. Ehrenberg, B. Jernström, F. Tjerneld, and B. Nordén, *Cancer Res.*, 1983, **43**, 1851.
3 M. K. Buening, P. G. Wislocki, W. Levin, H. Yagi, D. R. Thakker, H. Akagi, M. Koreeda, D. M. Jerina, and A. H. Conney, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 5358.
4 B. Jernström, P.-O. Lycksell, A. Gräslund, and B. Nordén, *Carcinogenesis*, 1984, **5**, 1129.
5 M. Eriksson, B. Jernström, A. Gräslund, and B. Nordén, *J. Chem. Soc., Chem. Commun.*, 1986, 1613.
6 J. B. Birks, 'Photophysics of Aromatic Molecules,' Wiley, Chichester and New York, 1980.
7 T. Meehan and K. Straub, *Nature*, 1979, **277**, 410.
8 M. Eriksson, B. Nordén, B. Jernström, and A. Gräslund, *Biochemistry*, in the press.