# OPTICALLY DETECTED MAGNETIC RESONANCE STUDY OF BENZO[a]PYRENE-7,8-DIHYDRODIOL 9,10-OXIDE COVALENTLY BOUND TO DNA

Steven M. LEFKOWITZ, Henry C. BRENNER, Donald G. ASTORIAN+ and Richard H. CLARKE+
*Department of Chemistry, New York University, New York, NY 10003* and *+Department of Chemistry, Boston University, Boston, MA 02215, USA*

Received 18 June 1979

## 1. Introduction

Recent investigations of the potent carcinogenic activity of benzo[a]pyrene have indicated that the major metabolite of this molecule responsible for in vivo binding to nucleic acids is a specific 7,8-dihydrodiol 9,10-oxide derivative [1–7] denoted by BPDE and shown in fig.1. The in vitro adduct, prepared by the reaction of BPDE and DNA [8], exhibits an extent of binding ~100-times greater than the in vivo product, making it more convenient for physico-chemical studies of the binding process. Evidence was recently obtained by fluorescence quenching [8,9] and electric field induced linear dichroism techniques [10] that in this in vitro adduct, the BPDE moiety, and in particular the pyrene-like chromophore, is not intercalated between the nucleic acid base pairs, but is instead located in a region external to the DNA helix. Other workers have asserted, however, that the pyrene-like chromophore is intercalated, based on the observations that BPDE causes unwinding of supercoiled DNA [11] and that the fluorescence of the chromophore is enhanced upon DNA denaturation [12].

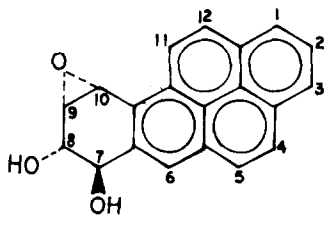



Fig.1. Structure of (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE).

Here we use the technique of optically detected magnetic resonance (ODMR) to probe the structure of the in vitro BPDE–DNA adduct. In this technique [13] the luminescence of the bound chromophore is monitored while the sample is irradiated with swept-frequency microwaves. Changes in both the phosphorescence and fluorescence intensities can generally be observed, when the microwave frequency is resonant with any one of the triplet zero field splittings. Using ODMR, one can measure not only the zero field splitting frequences but also the intersystem crossing rates to and from the triplet sublevels. Since these properties are all demonstrably sensitive to environment, ODMR is a potentially powerful probe of the interaction of carcinogens with specific sites in DNA. Here we apply the ODMR method to the BPDE–DNA adduct in an effort to further characterize the physical conformation at the binding site. Our measurements of the triplet zero field splittings and individual sublevel decay rates provide additional evidence that the pyrene-like chromophore of the BPDE moiety is not intercalated between the DNA base pairs, but is located on the outside of the DNA helix, exposed to the solvent.

## 2. Materials and methods

BPDE was kindly provided by Dr Ronald G. Harvey

of the University of Chicago. The BaP tetraol (7,8,9,10-tetrahydrobenzo[a]pyrene) was prepared by acid hydrolysis of BPDE. Calf thymus DNA was purchased from Worthington Chem. The in vitro covalent adduct of BPDE with DNA was prepared as in [8]. Unreacted and physically bound BPDE moieties were separated from the modified DNA by extracting the aqueous phase repeatedly with ether. The extent of binding was spectroscopically determined to be 5.8 BPDE molecules/1000 nucleotides. ODMR measurements were carried out on both the BPDE–DNA adduct ($5 \times 10^{-4}$ M nucleotide) and the BaP tetraol. Except where noted below, the solvent was a 1:1 (v/v) mixture of aqueous sodium cacodylate buffer and ethylene glycol.

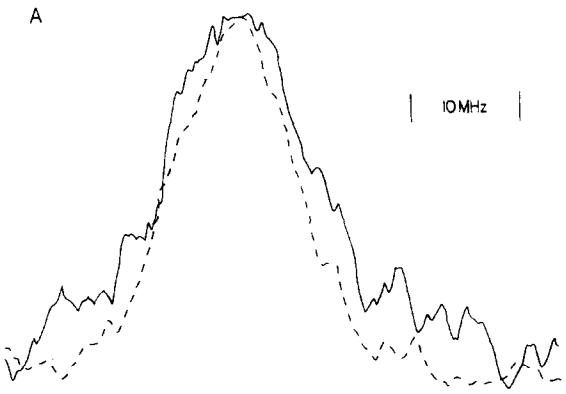
For the phosphorescence microwave double resonance (PMDR) studies, samples were cooled in quartz tubes to 2 K in a liquid helium cryostat, and excited optically by a 100 W Hg lamp whose output was passed through a Corning 7-54 filter. Phosphorescence emission was monitored at 598 nm with a 0.75 meter Jarrell Ash monochromator (slitwidth, 1000 $\mu$m; bandpass, 2.5 nm) and detected with a Bailey Centronics Q4283B photomultiplier tube. Phosphorescence was isolated from fluorescence and scattered light by a double blade chopper whose frequency was used as the reference signal for a PAR model 126 lock-in amplifier. The microwave source was a Hewlett-Packard 8690A sweeper with 8699B and 8692A plug-ins. A Hughes 1403H 1–2 GHz one watt amplifier was used in some runs to enhance the ODMR signals. Slow passage PMDR spectra were obtained by recording the phosphorescence signal on a Tracor Northern 570A signal averager, whose sweep was synchronized with the frequency sweep of the microwave source. The microwave frequency was swept at ~6 MHz/s; typically 500–1000 sweeps were required to obtain PMDR signals with reproducible linewidth and structure. A Hewlett Packard Model 540B transfer oscillator was used for microwave frequency measurements.

Details of the apparatus for fluorescence microwave double resonance (FMDR) have been reported [14]. FMDR spectra were obtained by monitoring the intensity of fluorescence at 399 nm. Values of the sublevel decay rates were derived by analyzing the response of the fluorescence intensity to square wave amplitude modulated microwaves at the zero field splitting frequencies [15].

## 3. Results and discussion

ODMR transitions were observed in the BPDE–DNA adduct at 0.997 ± 0.002 GHz, 2.036 ± 0.002 GHz and 3.032 ± 0.002 GHz. Since the aromatic chromophore in BPDE is pyrene, one expects the zero field splittings to be close to those measured for this molecule. The above values are within a few percent of the splittings measured for pyrene in a fluorene single crystal host [16] and 2-methyl tetrahydrofuran glass [17], and are distinctly removed from the splittings observed for benzo[a]pyrene in a variety of hosts [18].

To model environmental effects on the BPDE chromophore, we also studied the hydrolyzed tetraol of BPDE, which contains the same aromatic chromophore as BPDE but is less reactive in solution. Figure 2 compares the PMDR signals of the tetraol and the BPDE–DNA adduct. The similarity of the zero field splitting frequencies for the two molecules, each dissolved in the same solvent system, provides evidence that the pyrene chromophore is not intercalated, but is instead exposed to the solvent. The $D + E$ transition of the tetraol is nearly identical to that of the covalent complex, with respect to lineshape and frequency. The center frequencies of the $2E$ transitions are nearly the same, but the BPDE–DNA adduct does exhibit a broader, more structured line, which is suggestive of some conformational heterogeneity at the binding site. A similar heterogeneity was indicated in the fluorescence quenching [8] and linear dichroism [10] studies of this adduct. These ODMR signals are much narrower and show more detailed structure than those reported in recent ODMR studies of the covalent adducts of aflatoxin [19]



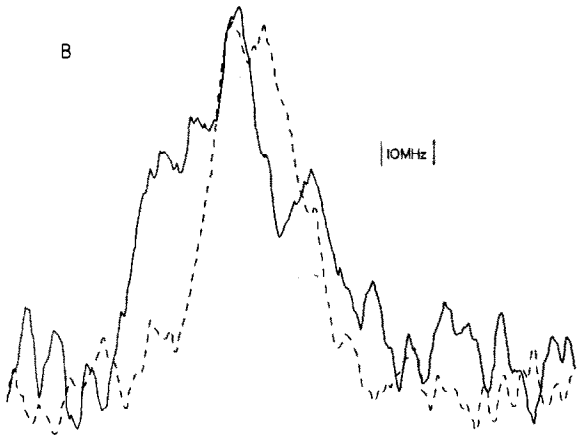



Fig.2. Phosphorescence microwave double resonance spectra at 2 K of the BPDE–DNA complex (——) and the BaP tetraol (- - - -), dissolved in ethylene glycol/buffer. A: $D + E$ transition. The microwave frequency was swept from 971 MHz (left) to 1021 MHz (right). B: $2E$ transition. The frequency was swept from 1991 MHz (left) to 2100 MHz (right). In both cases the sweep rates were slow enough (see text) so that when the sweep limits were reversed, the peak frequencies agreed to within 2 MHz.

and *N*-hydroxy-2-acetamidophenanthrene [20] with DNA.

The ODMR intensities appear to be strongly dependent on the choice of solvent and this provides further evidence that the pyrene chromophore is solvent exposed. If buffer alone was used as a solvent, no ODMR signals could be detected in the tetraol or the BPDE–DNA adduct, after extensive signal averaging.

Further information on the local environment of the bound chromophore can be obtained from the individual triplet spin sublevel decay rates. The triplet intersystem crossing rates have been shown to be highly sensitive to interactions with the surrounding medium; in particular, values for the sublevel decay rates for the case of polycyclic hydrocarbons physically bound (intercalated) to DNA have demonstrated this sensitivity in previous ODMR work [18,21]. The value of $k_z$, the decay rate for the spin sublevel aligned along the out-of-plane principal axis, has shown an anomalously large increase when benzo(a)pyrene [18,21] and benzanthracene [22] are intercalated in DNA. This finding is contrary to the expected behavior for the planar aromatic molecules in which $k_z$ is known to be the smallest decay rate in simple solvent environments [18,21,23]. For the BPDE–DNA adduct we measured by FMDR individual spin sublevel decay rates of $k_x = 2.0 \pm 0.2$, $k_y = 4.0 \pm 1.0$, and $k_z \simeq 0\ s^{-1}$, where the assignments for $x,y$ are arbitrary, but associated with the in-plane spin sublevels, and $k_z$ is the out-of-plane spin sublevel decay constant. Unlike the physically bound DNA complexes, the spin sublevel decay rates show the trend expected of polycyclic hydrocarbons in simple solvent systems, indicating that the local environment interactions are not those observed when polycyclic hydrocarbons are bound within the DNA base stack. These decay constants are also reasonably close to the values obtained for the pyrene triplet in a fluorene crystal host [16]. Furthermore, the average of the BPDE–DNA rates agrees well with our measurement of the overall triplet lifetime for the tetraol (0.48 s) measured from the phosphorescence decay at 77 K in the ethylene glycol/buffer solvent.

The above results indicate that ODMR will be a useful probe of the binding interactions of carcinogens with nucleic acids. Preliminary results [24] indicate that, in the covalent adduct of the weak carcinogen benzo[e]pyrene with DNA, the pyrene chromophore may be intercalated. ODMR should be useful in testing this hypothesis.

## Acknowledgements

We would like to acknowledge Dr R. G. Harvey for his generous gift of BPDE. Samples were prepared by Dr V. Ibanez. Dr Willem Leenstra provided experimental assistance in the initial parts of this work. Stimulating discussions with Professor N. E. Geacintov are gratefully acknowledged. This research was supported by grant CA 20851 to N. E. Geacintov and H. C. B. and grant CA 17922 to R. H. C. awarded by the National Cancer Institute, DHEW.

## References

[1] Borgen, A., Darvey, H., Castagnoli, N., Crocker, T., Rasmussen, R. and Wang, T. Y. (1973) J. Med. Chem. 16, 502.

[2] Sims, P., Grover, P. L., Swaisland, A., Pal, K. and Hewer, A. (1974) Nature 252, 326–327.
[3] Daudel, P., Duquesne, M., Vigny, P., Grover, P. L. and Sims, P. (1975) FEBS Lett. 57, 250–253.
[4] King, H. W. S., Osborne, M. R., Beland, F. A., Harvey, R. G. and Brookes, P. (1976) Proc. Natl. Acad. Sci. USA 73, 2679.
[5] Ivanovic, V., Geacintov, N. E. and Weinstein, I. B. (1976) Biochem. Biophys. Res. Commun. 70, 1172–1179.
[6] Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H. and Nakanishi, K. (1976) Science 193, 592–595.
[7] Jeffrey, A. M., Weinstein, I. B., Jennette, K. W., Grzeskowiak, K., Nakanishi, K., Harvey, R. G., Autrup, H. and Harris, C. (1977) Nature 269, 348–350.
[8] Prusik, T., Geacintov, N. E., Tobiasz, C., Ivanovic, V. and Weinstein, I. B. (1979) Photochem. Photobiol. 29, 223–232.
[9] Prusik, T. and Geacintov, N. E. (1979) Biochem. Biophys. Res. Commun. in press.
[10] Geacintov, N. E., Gagliano, A., Ivanovic, V. and Weinstein, I. B. (1978) Biochemistry 17, 5256–5262.
[11] Drinkwater, N. R., Miller, J. A., Miller, E. C. and Yang, N. C. (1978) Cancer Res. 38, 3247–3255.
[12] Yang, N. C., Ng, L., Neoh, S. B. and Leonov, D. (1978) Biochem. Biophys. Res. Commun. 82, 929–934.
[13] Kwiram, A. L. (1972) in: MTP Intn'l Rev. Sci. Phys. Chem. ser. I; Magnetic Resonance (McDowell, C. A. ed) vol. 4, pp. 271–316, Univ. Park Press, Baltimore.
[14] Clarke, R. H. and Hofeldt, R. H. (1974) J. Chem. Phys. 61, 4582–4587.
[15] Chiha, P. A. and Clarke, R. H. (1978) J. Mag. Res. 29, 535–543.
[16] Sixl, H. and Schwoerer, M. (1970) Z. Naturforsch. 25a, 1383–1394.
[17] Brinen, J. S. and Orloff, M. K. (1966) J. Chem. Phys. 45, 4747–4750.
[18] Chiha, P. A., Clarke, R. H. and Kramer, E. (1977) Chem. Phys. Lett. 50, 61–64.
[19] Kwiram, A. L., Liu, Y. C., Farquhar, M. N. and Smuckler, E. A. (1978) Biochem. Biophys. Res. Commun. 83, 1354–1359.
[20] Co, T-t., Liu, Y. C., Poling, M., Schriber, J. D. and Kwiram, A. L. (1979) in preparation.
[21] Chiha, P. A., Clarke, R. H., Kramer, E. and Lasser, N. (1978) Semicond. Insul. 4, 377–392.
[22] Clarke, R. H. and Lasser, N. (1979) unpublished.
[23] Clarke, R. H. and Frank, H. A. (1976) J. Chem. Phys. 65, 39–47.
[24] Geacintov, N. E., Ibanez, V., Gagliano, A. G., Harvey, R. G. and Lee, H. M. (1979) in preparation.