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Stereoselective Covalent Binding of Enantiomers of *anti*-Benzo[*a*]pyrene Diol Epoxide to DNA As Probed by Optical Detection of Magnetic Resonance†

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ABSTRACT: Phosphorescence and optical detection of magnetic resonance measurements applied to the covalent adducts of (+)- and (−)-*anti*-benzo[*a*]pyrene with DNA show a marked red shift of the pyrenyl phosphorescence and a lowering of the zero field splitting parameters of the (−) adduct, relative to the (+) adduct and the (solvent-exposed) benzo[*a*]pyrene tetraol. These results are consistent with a predominance of quasi-intercalative sites in the (−) adduct and external, solvent-exposed sites in the (+) adduct.

The two enantiomers of *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BaPDE,[1] Figure 1) are characterized by remarkably different tumorigenic and mutagenic activities (Conney, 1982; Harvey, 1981). The biological effects of these (and other) reactive polycyclic aromatic hydrocarbon metabolites are believed to involve the formation of adducts derived from the covalent binding of BaPDE to DNA (Singer & Grunberger, 1983; Brookes & Osborne, 1982). It is therefore of great interest to determine if there are any significant differences in the conformations and properties of the adducts derived from the binding of the (+) and (−) enantiomers of BaPDE to double-stranded DNA, which might ultimately provide an insight into the biochemical activities of these compounds on a molecular level.

In the last few years, optical detection of magnetic resonance (ODMR) has been employed as a probe of the structure of complexes of DNA with polycyclic aromatic hydrocarbon carcinogens (Chiha et al., 1977, 1978; Lefkowitz et al., 1979; Lefkowitz & Brenner, 1981, 1982; Clarke et al., 1983). As is well-known, the excited triplet state of an organic molecule

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[1] Abbreviations: BaPDE, *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BaPT, 7,8,9,10-tetrahydroxytetrahydrobenzo[*a*]pyrene; BeP, benzo[*e*]pyrene; BePE, 9,10-epoxy-9,10,11,12-tetrahydrobenzo[*e*]pyrene; DNA, deoxyribonucleic acid; EGB, ethylene glycol/buffer solution; GHz, gigahertz; MHz, megahertz; ODMR, optically detected magnetic resonance.

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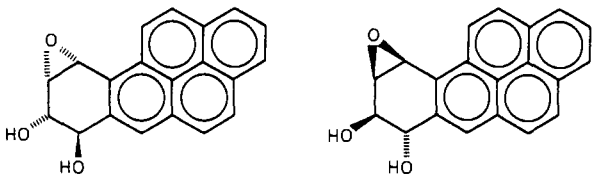

FIGURE 1: The (+) and (−) enantiomers of *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BaPDE).

is split into three nondegenerate magnetic sublevels by the dipolar interaction of the unpaired electron spins. In the ODMR technique (Kwiram, 1972), the magnetic sublevels are pumped with resonant microwaves while the phosphorescence from the triplet chromophore is observed; since the spin sublevels generally have distinct rate constants for phosphorescence, owing to selective spin-orbit coupling, a change in phosphorescence intensities can generally be produced at resonance. Since the zero field splitting parameters are demonstrably sensitive to molecular environment, the ODMR technique can be a useful probe of the structure of DNA–carcinogen adducts, particularly when comparisons with appropriate model systems are made.

Previous spectroscopic studies of DNA adducts of benzo[*a*]pyrene epoxides have implied the existence of two main classes of binding sites, defined in terms of the degree of interaction of the pyrenyl chromophore with the DNA bases (Geacintov et al., 1982). In site I structures, there is appreciable intercalative or base stacking interaction with the aromatic chromophore; site I adducts have been termed "quasi-intercalative", since they clearly show base stacking interactions with the PAH chromophore, without necessarily exhibiting a classical intercalation geometry. In site II structures, by contrast, the chromophore shows little interaction with the bases and is apparently solvent exposed. These classes of binding sites show distinctive properties in terms of shifts of UV absorbance, chromophore orientation as determined by linear electric dichroism, and differing fluorescence yields (Geacintov, 1985). Previous ODMR studies (Lefkowitz & Brenner, 1982) of DNA adducts of 9,10-epoxy-9,10,11,12-tetrahydrobenzo[*e*]pyrene (BePE) have suggested that the triplet zero field splittings of site I (quasi-intercalative) adducts should generally be shifted down in frequency relative to those of solvent-exposed adducts. In this paper, we apply ODMR to the DNA adducts of the (+) and (−) enantiomers of *anti*-BaPDE [referred to here as simply (+)- and (−)-BaPDE] and find that the complexes show quite dramatically different phosphorescence and zero field splittings, implying that the conformations of the two types of adducts are quite different. The results support the correlation between base stacking interactions and red shifts in phosphorescence and zero field splittings and provide further evidence for site I conformations of (−)-BaPDE adducts and predominantly site II structure for the adducts derived from the binding of (+)-BaPDE to DNA (Geacintov et al., 1984; Jernstrom et al., 1984).

Materials and Methods

Sample Preparation. The epoxides (+)- and (−)-BaPDE were obtained from the NCI Carcinogen Standard Reference Repository, Bethesda, MD. Calf thymus DNA was purchased from Sigma Chemical Co. The procedure for preparation of the covalent DNA adducts has been described previously (Prusik et al., 1979). Basically, the epoxide of interest was dissolved in tetrahydrofuran (ca. 0.01 M), and aliquots of this solution were added to DNA in aqueous sodium cacodylate buffer (5 mM); the mixture was incubated at 25 °C for 1 h. The modified DNA was then extracted with ether and reprecipitated with cold ethanol to remove unreacted or hydrolyzed epoxides. The adducts were then redissolved in 5 mM cacodylate buffer (pH 7). The degree of modification (BaPDE bound per base pair), as determined by UV absorbance measurements, was ca. 3% for (+)-BaPDE and 0.3% for (−)-BaPDE. Typically, the DNA concentration of the samples was 0.7 mM, expressed in concentration of nucleotides. Because of the sensitivity of the adducts to near-UV irradiation (Zinger et al., 1987; Zinger, 1986), the samples were repeatedly extracted with ethyl ether until no fluorescence due to dissociated pyrenyl residues (essentially due to the tetraol 7,8,9,10-tetrahydroxytetrahydrobenzo[*a*]pyrene, BaPT) (Zinger et al., 1987) could be detected in the washings, before ODMR or phosphorescence measurements were performed.

Denaturation of DNA adducts was accomplished by heating an aliquot of the original solution of adducts in a boiling water bath for 20 min, followed by rapid cooling in an ice–water bath. Samples were then extracted with ethyl ether to remove any dissociated aromatic residues. Enzymatic digestion to individual modified deoxyribonucleosides was done according to a scheme in which the modified DNA was treated successively with DNase I (Sigma), alkaline phosphatase (Sigma), and spleen and snake venom phosphodiesterase I and II (Sigma) and incubated at 37 °C for 22 h (Brown et al., 1979). The modified deoxyribonucleosides were then separated from unmodified monomers by Sephadex LH-20 chromatography.

The tetraol BaPT, used to model solvent-exposed environments, was obtained from the washings of the DNA adduct solutions. The BaPT concentration in the solutions used in the experiments was typically 0.01 mM.

Phosphorescence and ODMR. For low-temperature experiments, about 0.1 mL of the adduct (native, denatured, or digested) or tetraol in cacodylate buffer solution was mixed with an equal volume of ethylene glycol (Fisher Scientific). This solvent is useful for producing a good optical-quality glass at low temperatures; furthermore, the presence of ethylene glycol is essential for ODMR signals to be detected (Kolubayev, 1986). The ethylene glycol/buffer (EGB) solution was then transferred to a Suprasil quartz sample tube and quickly frozen to 77 K. Phosphorescence measurements were then performed, or the sample was cooled further for the ODMR measurements. It should be noted that samples which had been allowed to warm to room temperature were never reused. This was to avoid, as much as possible, experimental artifacts, which might arise from the presence of BaPT photodecomposition products in the samples (Zinger et al., 1987; Zinger, 1986).

The procedures for recording 77 K phosphorescence and ODMR spectra at 2 K have been reported previously (Lefkowitz et al., 1979). Briefly, samples were irradiated by the filtered output of a 100-W Hg lamp, and the phosphorescence was isolated with a rotating double-blade chopper phosphoroscope, used in combination with a lock-in amplifier. To detect ODMR, the amplified (1-W) output of a Hewlett-Packard microwave sweeper was frequency-swept while the phosphorescence maximum of the pyrenyl chromophore was viewed at ca. 600 nm. The frequency sweeps were synchronized with the sweeps of a Tracor-Northern 570A signal averager. Typical ODMR runs required several hundred sweeps of ca. 10 s each through the frequency range of interest.

Results and Discussion

Table I presents the phosphorescence emission maxima and ODMR peak frequencies for (+)- and (−)-BaPDE–DNA adducts. It should be mentioned that, owing to limited sample

Table I: Phosphorescence and ODMR Frequencies for (+)- and (−)-BaPDE–DNA Adducts and Model Systems[a]

sample	phos. max (nm)	$D + E$ (MHz)	$2\|E\|$ (MHz)
BaPT	600	990 ± 2	2046 ± 3
(+)-BaPDE–DNA	600	992 ± 2	2036 ± 3
(−)-BaPDE–DNA	610	986 ± 5	2000 ± 3
(+) denatured	605	980 ± 5	2015 ± 4
(−) denatured	605	982 ± 2	2019 ± 5
(±) digest[b]	600	990 ± 2	2038 ± 4
(−) digest	600	990 ± 4	2040 ± 5

[a] All samples were studied in 1:1 by volume ethylene glycol/cacodylate buffer solvent. Phosphorescence maxima (estimated precision ±1 nm) were recorded at 77 K, while ODMR data were obtained at 1.6 K. [b] Digest of racemic adduct; see text.

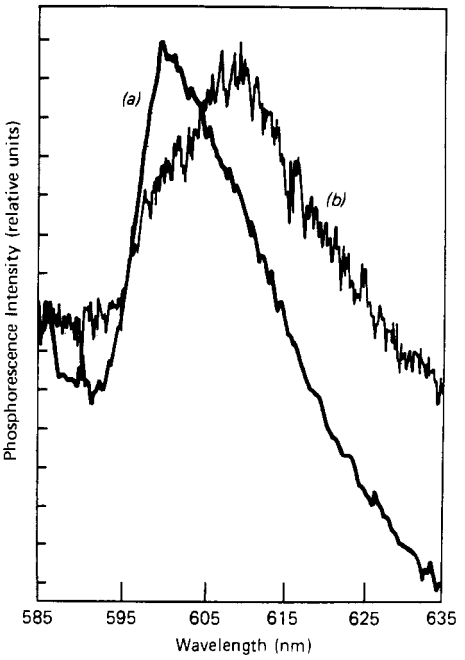

FIGURE 2: Phosphorescence emission spectra at 77 K of the covalent DNA adducts of (+)-BaPDE (curve a) and (−)-BaPDE (curve b). The base line of curve b has been displaced for ease of comparison.

availability, a digest of the racemic *anti*-BaPDE was studied rather than a digest of the (+) adduct. The properties of the digest of the racemic adduct should be very similar to those of a digest of the (+) adduct, since it is known (Meehan & Straub, 1979) that (+)-BaPDE binds up to 7 times more extensively to DNA than does the (−) enantiomer. Thus adducts formed from racemic *anti*-BaPDE are mostly due to the (+) enantiomer. Studies of samples prepared from (+) and racemic *anti*-BaPDE have shown very similar phosphorescence and ODMR properties (Kolubayev, 1986).

The first aspect to note in Table I is the dramatic contrast between the phosphorescence emission maxima and the positions of the ODMR transitions of the (+)- and (−)-BaPDE–DNA covalent adducts. The phosphorescence emission maximum of the (−) adduct is red-shifted by a full 10 nm relative to that of the (+) adduct (Figure 2); moreover, the ODMR frequencies, particularly the $2|E|$ value, are substantially lower (Figure 3). If one associates red shifts in phosphorescence and zero field splittings with quasi-intercalative binding, as suggested by our earlier work on 9,10-epoxy-9,10,11,12-tetrahydrobenzo[*e*]pyrene (BePE) (Lefkowitz & Brenner, 1982), then this would implicate predominantly site I binding in the (−) adduct. This agrees qualitatively with the conclusions of recent workers (Geacintov et al., 1984) who find by means of linear electric dichroism and UV absorbance shifts

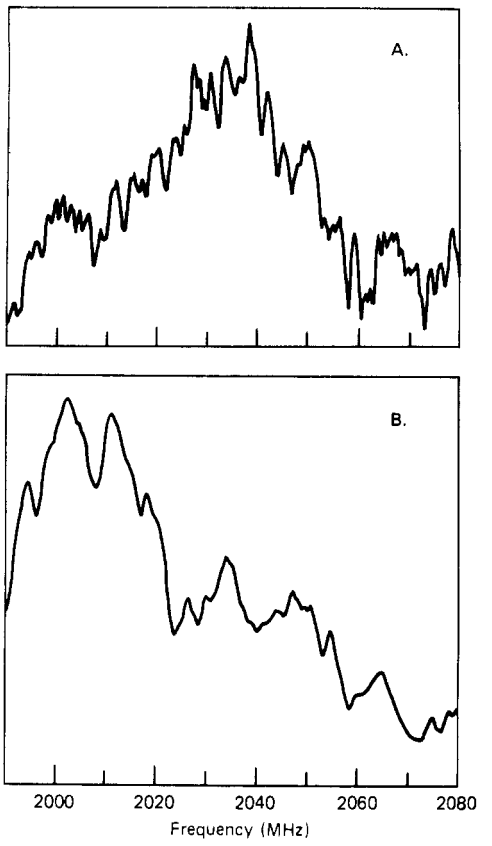

FIGURE 3: The $2|E|$ ODMR spectra at 1.6 K of the covalent DNA adducts of (+)-BaPDE (panel A) and (−)-BaPDE (panel B). For the (+) adduct, phosphorescence was monitored at 600 nm, and ca. 300 sweeps of 20-s duration were accumulated. In the case of the (−) adduct, phosphorescence was viewed at 610 nm, and ca. 650 sweeps of 15 s each were required. Other spectra (not shown) confirm the fall-off to base line below 1990 MHz implied in panel B.

that the major site for binding in the (−) adduct is quasi-intercalative in nature, while the (+) adduct shows external binding. Additional support for site II binding in the (+) adduct comes from the observation that the (+) adduct phosphorescence and ODMR are only slightly shifted from the values found for the tetraol in EGB, which is our model for complete solvent exposure.

In our previous work, we used denaturation and enzymatic digestion as probes for the existence of quasi-intercalative binding in the BePE-DNA adduct (Lefkowitz & Brenner, 1982). We observed that the ODMR transitions shifted upward in frequency when the adducts were denatured and digested. This was taken as additional evidence for quasi-intercalative binding in the original native adduct. In this work we find that, while the phosphorescence emission maximum of the (−)-BaPDE adduct exhibits a blue shift upon denaturation, the maximum in the (+) adduct is actually red shifted, relative to its position in the original native DNA adduct. The phosphorescence and ODMR data for the two denatured adducts now agree rather closely with one another, suggesting that the environment of the pyrenyl chromphore is similar in the two types of complexes. The spectroscopic parameters are still substantially red shifted, relative to the tetraol, however. This could be taken as evidence for some pyrenyl residue–nucleic acid base stacking interactions in single-stranded DNA. For example, it has been shown (Nelson & De Voe, 1984) by UV absorbance shifts that pyrene can form physical complexes with denatured DNA quite readily. It is also observed (N. E. Geacintov, D. Zinger, V. Ibanez, R. Santella, D. Grunberger, and R. G. Harvey, unpublished work) that the 346-nm

absorbance maximum of the pyrenyl chromophore in the native DNA adduct of racemic *anti*-BaPDE is red-shifted to 351 nm when the DNA is subjected to denaturation. This also suggests an increase in carcinogen base stacking in the denatured form.

It should be noted that, in our previous studies of DNA complexes with racemic BaPDE (Lefkowitz & Brenner, 1982), denaturation was reported to produce no shift in phosphorescence or ODMR. We would have expected to see results similar to our results for the (+) adduct presented here, since, as noted above, DNA adducts prepared with racemic *anti*-BaPDE are comprised mostly of (+) adducts. These previous observations may have been caused by the presence of tetraol dissociation products and/or incomplete denaturation of the DNA. As reported recently (Zinger et al., 1987), these dissociation products can dominate the luminescence properties of samples that are nominally composed of BaPDE–DNA adducts.

A more conclusive test for the presence of quasi-intercalative binding sites in native DNA is the digestion to individual deoxyribonucleosides. As expected, in the case of the deoxyribonucleoside adducts, the positions of the phosphorescence emission maxima and the ODMR frequencies are now nearly identical with those of the solvent-exposed tetraol (Table I). This is consistent with the presence of carcinogen–base stacking binding sites (site I) in the original native (−) adduct, which are characterized by a low degree of solvent exposure.

Thus, assuming that red shifts in phosphorescence and ODMR can be correlated with quasi-intercalative binding, we conclude that the (+)-BaPDE adducts show predominantly external, solvent-exposed binding, while the major binding site in (−)-BaPDE–DNA adducts is quasi-intercalative. It is not possible to gauge the degree of intercalative interaction in the (−) adduct; however, the carcinogen–base stacking interactions, as determined from the phosphorescence emission red shifts and ODMR resonances, are probably stronger than those in the BePE–DNA complex. In the case of these latter complexes, we observed a lowering of the zero field splittings relative to those observed in the case of the solvent-exposed BeP diol (Lefkowitz & Brenner, 1982) but no optical red shift in the phosphorescence itself. The inference that site I binding dominates in (−)-BaPDE–DNA adducts, while site II binding dominates in the (+) adducts, agrees with the conclusions of other recent spectroscopic studies performed in our laboratory (Geacintov et al., 1984) and elsewhere (Jernstrom et al., 1984; Chen, 1985). Thus, we conclude that ODMR can be quite useful as a probe of the structure of DNA–carcinogen adducts, when used in combination with other spectroscopic techniques. ODMR studies of DNA complexes of 1-oxyranylpyrene and 9,10-epoxybenzo[*a*]pyrene (Kolubayev, 1986) have reinforced this conclusion.

Finally, the higher biological activity of (+)-BaPDE relative to (−)-BaPDE has been attributed to spatial differences in the orientations of the covalent adducts formed (Brookes & Osborne, 1982). Hingerty and Broyde have proposed that the (−)-BaPDE–DNA adducts, because of their tendency to form intercalative site I adducts that cause a severe local distortion of the native DNA structure, are more easily recognized and excised by mammalian repair enzymes than the external site II (+)-BaPDE–DNA adducts, which cause relatively little deformation in the DNA structure (Hingerty & Broyde, 1985).

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