Characterization of Reversible, Physical Binding of Benzo[a]pyrene Derivatives to DNA*

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The carcinogen, 7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, and a number of its noncarcinogenic derivatives form a reversible physical complex with DNA in vitro. The absorbance spectrum of the hydrocarbon in the complex shifts ~10 nm to the red. Fluorescence from the physically bound hydrocarbon is significantly quenched, and binding levels are decreased by the addition of 1) spermine, 2) MgCl₂, and 3) NaCl, with their relative effectiveness in reducing complex formation following the same order. Equilibrium data and the change in superhelicity of simian virus 40 DNA as a result of physical binding were used to calculate an unwinding angle for each of the benzo[a]pyrene derivatives of 13°, while under the same conditions ethidium bromide unwound the duplex ~30°. The weight of this evidence indicates that the carcinogen and its noncarcinogenic derivatives reversibly bind to DNA by intercalation of their planar aromatic system into the stacked base pairs of the duplex. The results of this and previous studies suggest that covalent binding of the carcinogenic derivative of benzo[a]pyrene to DNA is preceded by a physical intercalation step.

BP is a potent carcinogen and widespread environmental contaminant. A number of studies (Brookes and Lawley, 1964; Gelboin, 1965; Heidelberger, 1975) have shown that this relatively inert polycyclic aromatic hydrocarbon must undergo metabolic conversion to its biologically active form, BPDE (Sims et al., 1974; Huberman et al., 1976; Meehan et al., 1976; Weinstein et al., 1976; Koreeda et al., 1976; King et al., 1976). Previous reports have shown that a racemic mixture of the BPDE reacts with the exocyclic amino groups of guanine, adenine, and probably cytosine with nucleic acids both in vivo and in vitro (Weinstein et al., 1976; Meehan et al., 1977; and Ivanovic et al., 1978). Guanine is the predominant alkylation site and reaction of the racemic BPDE at this position was shown to occur stereoselectively (Meehan and Straub, 1979). The asymmetric reaction is dependent on double-stranded DNA and the higher level of binding obtained with the (+)-enantiomer correlates well with its greater mutagenic and carcinogenic potency (Wood et al., 1977; Buening et al., 1978). The detailed mechanism of this reaction is unknown. We have suggested that the hydrocarbons could form asymmetric complexes by physically intercalating into DNA prior to covalent binding, with the result that the (+)-enantiomer is in a more favorable position to alkylate the guanine binding site (Meehan and Straub, 1979).

A number of planar drugs and dyes undergo reversible, physical intercalation into DNA in which the planar compound inserts between the base pairs and is stabilized primarily by hydrophobic interactions (Waring, 1981). X-ray crystal structures have been obtained for oligonucleotide complexes formed with proflavin (Neidle et al., 1977), 9-aminooacididine (Seeman et al., 1975), a terpyridine platinum compound (Wang et al., 1978), ethidium bromide (Tsai et al., 1977), and daunomycin (Quigley et al., 1980). Although this form of DNA interaction is well established for water-soluble, usually charged, dyes, few examples exist among water insoluble, neutral hydrocarbons, such as BPDE. A recent report (Geacintov et al., 1981) has shown that BPDE physically binds to DNA and it was suggested that the hydrocarbon in this complex may be intercalated. We have undertaken a detailed analysis of the physical complex formed between BPDE and DNA in order to assess the nature of this interaction. As a result of the instability of BPDE in aqueous solvents (Kellar et al., 1976; Yang et al., 1977; Whalen et al., 1979), it is not possible to obtain direct structural information concerning the complex by x-ray crystallography. However, the indirect evidence we have accumulated, when taken together, establishes that the physical complex formed between BPDE and DNA results from intercalation. Preliminary accounts of this work have been presented, (Meehan et al., 1981).

Our approach utilized optical measurements and a determination of the helix unwinding angle after binding of the hydrocarbon to relaxed closed circular DNA. Several structurally related analogs of the BPDE were investigated and were also found to physically intercalate into DNA. These compounds lack a 9,10-double bond rendering them noncarcinogenic (Levin et al., 1978). We chose conditions under which the hydrocarbons would bind minimally to the outside of the helix and our evidence showed that the major interaction between BPDE and DNA was intercalation.

MATERIALS AND METHODS

**Chemicals, Synthesis, and Supplies**—SV40 DNA was purified from infected monkey kidney cells (Hallick et al., 1978) or obtained from Bethesda Research Laboratories. The superhelical content was >95%.

1 The abbreviations and trivial name used are: BP, benzo[a]pyrene; BPDE, racemic 7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; (also referred to as anti-BPDE); 7-(OH)H₂BP, 7-hydroxy-7,8,9,10-tetrahydroBP; 8,9,10-tri(OH)H₂BP, 8,9,10-tri(OH)BP; trans-9,10-dihydroxy-7,8,9,10-tetrahydroBP; cis-7,8-dihydroxy-7,8,9,10-tetrahydroBP; tel-trol, 7,8,9,10-tetrahydroBPE; HPLC, high performance liquid chromatography; SV40, simian virus 40; r, ratio of bound hydrocarbon to DNA base pairs; THF, tetrahydrofuran.

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as evidenced by agarose gel electrophoresis. Calf thymus DNA was purchased from Sigma. This preparation was highly polymerized, as evidenced by agarose gel electrophoresis, and contains a high degree of secondary structure since we obtained hyperchromicities > 38%. The BPDE was synthesized by literature methods (McCaustland and Engle, 1975; Yagi et al., 1975) and characterized by UV, visible, and fluorescence spectra and by base composition. DNA was sonicated into 20 ml of water containing DNA at 44 wavelengths for 1 h.

Relaxation Measurements—Spectra were taken in the presence of DNA (Geacintov et al., 1980). Rates of solvolysis were measured spectrophotometrically by taking advantage of the 2 nm spectral difference between the tetrol and the BPDE (Kellar et al., 1975). Spectral measurements were made within 30 s of mixing the hydrocarbon into an aqueous solution, by baseline resolution, at a concentration of 5 mg/ml, and 25-ml aliquots were made. The unwinding angle measurements required 10-min exposures of the BPDE to an aqueous environment at 25 °C and under these conditions the hydrocarbon had a t1/2 of 70 min. Thus, even at the end of the relaxation reaction < 7% of the BPDE was converted to solvolysis products.

Relaxation of SV40 DNA—Relaxation of superhelical DNA was catalyzed by a homogeneous preparation of calf thymus topoisomerase. The enzyme was supplied by Dr. James Wang (Harvard University) and was diluted just prior to use in 50 mM potassium phosphate, pH 7.4, containing 1 mM EDTA, 0.5 mM dithiothreitol and 10% glycerol. Relaxations with bound BPDE were carried out in 0.01 M NaHCO3, pH 9.0, containing 10% acetone and either 50 or 200 mM NaCl (all other BP derivatives were tested under the same conditions, as indicated). 0.4 pg of SV40 DNA was relaxed with 1 μl of the enzyme. Relaxation of SV40 DNA by topoisomerase results in a Gaussian distribution of molecules differing by integral values of α and, since β is constant, integral values of β. The distribution can be seen after high resolution gel electrophoresis of a relaxed sample. The Gaussian is centered closely about a completely relaxed species. The average superhelix density (γ) of each sample was determined from a densitometry tracing of the appropriate gel. Since γ is relative in a control to a control equal is the change in the binding of the BPDE and the number of hydrocarbons bound is known from our equilibrium measurements an unwinding angle is readily calculated.

Equilibrium Constants—The equilibrium constants (Keq) for binding of hydrocarbon to DNA were obtained by an approach originally reported by Benesi and Hildebrand (1949) and more recently adapted by Schmechel and Crothers (1971). When binding measurements are carried out under conditions where we can write

\[ \frac{C_{B}}{C_{R}} > C_{T} \]

\[ K_{eq} = \frac{C_{D}}{C_{B}} \]

where \( C_{B} \) = [hydrocarbon, bound], \( C_{R} \) = [hydrocarbon, free], \( C_{D} \) = [total nucleotide, in base pairs], \( C_{H} \) = [hydrocarbon, total] = \( C_{B} \) + \( C_{R} \), and \( r = C_{B}/C_{D} \), and

\[ \lim_{t \to 0} K_{eq} = K_{in} \]

The absorbance spectra of BPDE in the presence and absence of DNA. BPDE in THF was added to give a concentration of 5 μM [final concentration THF, 15%] to a cuvette containing 0.01 M NaHCO3, pH 9.0, 10% acetone equilibrated at 21 °C. The sample was mixed and an absorbance spectrum recorded immediately in the wavelength range 300-400 nm (curve 1). The same procedure was repeated but the buffer contained in addition 3.8 mg/ml of sonicated calf thymus DNA (curve 2). The spectrum is shown in the presence of DNA approaches total binding of the hydrocarbon, since [DNA] >> BPDE. A sample containing two intermediate levels of binding exhibits a composite spectrum of curves 1 and 2. Curve 3 represents the sample in curve 2 minus BPDE.

Fig. 1. The absorbance spectra of BPDE in the presence and absence of DNA. BPDE in THF was added to give a concentration of 5 μM [final concentration THF, 15%] to a cuvette containing 0.01 M NaHCO3, pH 9.0, 10% acetone equilibrated at 21 °C. The sample was mixed and an absorbance spectrum recorded immediately in the wavelength range 300-400 nm (curve 1). The same procedure was repeated but the buffer contained in addition 3.8 mg/ml of sonicated calf thymus DNA (curve 2). The spectrum is shown in the presence of DNA approaches total binding of the hydrocarbon, since [DNA] >> BPDE. A sample containing two intermediate levels of binding exhibits a composite spectrum of curves 1 and 2. Curve 3 represents the sample in curve 2 minus BPDE.
From these relationships, the following can be derived (Schmechel and Crothers, 1971).

\[ \frac{1}{e_{\text{app}} - e_{\text{f}}} = \frac{1}{(e_{\text{f}} - e_{\text{0}})(C_{\text{app}} - C_{\text{f}})K_{\text{app}}} + \frac{1}{e_{\text{f}} - e_{\text{0}}} \]

where \( e_{\text{app}} \) = apparent extinction coefficient, \( C_{\text{f}} + C_{\text{r}}; e_{\text{f}} = \) extinction coefficient, \( C_{\text{r}}; e_{\text{0}} = \) extinction coefficient, \( C_{\text{0}}. \) A plot of \( \frac{1}{e_{\text{app}} - e_{\text{f}}} \)

versus \( \frac{1}{C_{\text{app}} - C_{\text{f}}} \) results in a linear relationship whose slope and intercept yield the necessary values to determine \( K_{\text{app}}. \) The conditions used to determine \( K_{\text{app}} \) were the same as used for the SV40 DNA relaxations.

Instrumentation—Absorbance measurements and spectra were recorded on an Amino DW-2a spectrophotometer (American Instrument Co., Silver Spring, MD). Fluorescence measurements and spectra were recorded on a Baird-Atomic Fluorocord (Braintree, England). HPLC was carried out on an Altex system (Berkeley, CA) fitted with model 100A pumps, a Schoeffel fluorescence detector, and a Hitachi (model 10-40) variable wavelength absorbance detector. Silica columns were either 25 cm x 9.4 mm or 25 cm x 4.3 mm and were obtained from Whatman or Dupont (Wilmington, DE). The purity of all hydrocarbons used was confirmed by HPLC on silica columns using tetrahydrofuran:hexane as eluents.

### RESULTS

**Spectral Shift of BPDE Bound to DNA**—In the presence of calf thymus DNA at pH 9.0 the absorbance spectrum of the BPDE shifts -10 nm to the red (Fig. 1), indicating formation of a physical complex. Under these same conditions we find that covalent binding of BPDE to DNA exhibits a \( \tau_{1/2} \approx 60 \) min and therefore does not contribute to the shifted peak. The reversible nature of physical complex formation is indicated by a reduction in its absorbance upon the addition of certain salts (see below). Furthermore, we find the absorbance of the complex decreases at the same rate as BPDE hydrolysis, reflecting the lower intercalation level of the solvolysis product (see equilibrium constants, Table I). A similar observation has also been reported (Geacintov et al., 1981). The BPDE-DNA spectrum displayed in Fig. 1 was obtained under conditions where DNA was in large excess and, therefore, nearly all the hydrocarbon was bound. When spectra were recorded with various concentrations of DNA (and constant BPDE), a series of curves were obtained with isosbestic points, indicating the probable presence of only two hydrocarbon species, bound and free. Although this type of spectral behavior is characteristic of intercalated dyes it cannot be taken as proof of intercalation (Bloomfield et al., 1974). It does, however, demonstrate the formation of a physical complex between the BPDE and DNA as was recently reported (Geacintov et al., 1981).

### Table I

**Equilibrium constants of BPDE and its derivatives**

For each measurement the hydrocarbons were added in 10 μl of tetrahydrofuran to a cuvette containing final concentrations of 0.01 mM NaHCO₃, 10% acetone, 200 mM NaCl, and Mg²⁺ where indicated; the pH was 9.0. The samples were mixed and readings taken within 30 s for those containing BPDE. Absorbance measurements were made at 354 nm as a function of DNA concentration (0.35-3.8 mg/ml) and the data obtained used to calculate \( K_{i} \), as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temperature</th>
<th>Mg²⁺</th>
<th>( K_{i} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td>mM</td>
<td>M⁻¹</td>
</tr>
<tr>
<td>BPDE</td>
<td>25</td>
<td>0</td>
<td>551</td>
</tr>
<tr>
<td>BPDE</td>
<td>25</td>
<td>20</td>
<td>753</td>
</tr>
<tr>
<td>7,8-di(OH)H,BP</td>
<td>25</td>
<td>8</td>
<td>514</td>
</tr>
<tr>
<td>9,10-di(OH)H,BP</td>
<td>8</td>
<td>355</td>
<td></td>
</tr>
<tr>
<td>7-(OH)H,BP</td>
<td>8</td>
<td>2</td>
<td>319</td>
</tr>
<tr>
<td>Tetrrol</td>
<td>8</td>
<td>2</td>
<td>88</td>
</tr>
</tbody>
</table>

* Conditions are the same as listed above but with 50 mM NaCl.

Salt Dissociation of the Complex—The presence of various cations affected the amount of physical complex formed as demonstrated by the change in absorbance at 354 nm, shown in Fig. 2. The complex dissociates in the presence of the indicated salts and the relative extent of dissociation on a molar basis is spermine > MgCl₂ > NaCl. This is the same order of efficiency that these agents exhibit in neutralizing the negative charge on DNA. Charge neutralization leads to a more compact structure and, thus, results in a decrease in the average distance between base pairs. This could have the effect of decreasing the number of intercalation sites available in the polymer. Other explanations are possible for this phenomenon, e.g. salt interference with binding of the hydrocarbon to the outside of the helix, but the latter possibility is less likely (see below).

Equilibrium Binding Constants—Table I presents the \( K_{i} \) values obtained for the BPDE and a number of its stable derivatives. The BPDE had a larger binding constant than any of the derivatives tested. The overall binding process is exothermic and, therefore, we carried out \( K_{i} \) measurements on the BPDE at 25 °C while the remainder of the hydrocarbons were measured at 8 °C. This resulted in similar binding levels for each compound tested (with the exception of tetrrol). The relatively low binding levels for all of the hydrocarbons compared to water-soluble intercalating agents was due in part to the presence of 10% acetone in the solvent. The acetone produced two competing effects; it served to increase the solubility of the derivatives while binding was decreased due to sequestering of the hydrocarbons. There is a 5-fold difference in the binding levels of the BPDE and tetrrol and this difference would be larger if binding were measured at the same temperature. Since binding levels are almost the same in the 9,10-di(OH) and 7,8-di(OH) analogs, the difference in BPDE and its solvolysis product is probably related to solubility and not conformational differences.

Fluorescence of BPDE-DNA Complex—BP derivatives ex-
hibit characteristic fluorescence spectra (Meehan et al., 1976). The fluorescence excitation of BPDE at 345 nm was reduced 40% in the presence of DNA compared to the hydrocarbon in the absence of DNA. From equilibrium data, 52% of the hydrocarbon would actually be physically bound. If we assume the fluorescence loss is due to binding, then 77% of the fluorescence is quenched by this process. Due to uncertainties in the binding measurements it is conceivable that fluorescence from the complexed hydrocarbon is totally quenched. Some intercalating agents such as the complexed hydrocarbon. Therefore, the fluorescence of the absence of DNA. From equilibrium data, the fluorescence excitation of BPDE at the fluorescence of the complexed hydrocarbon is totally quenched. Due to uncertainties, the fluorescence loss is due to binding, then 77% of the fluorescence is quenched by this process. Due to uncertainties in the binding measurements it is conceivable that fluorescence from the complexed hydrocarbon is totally quenched. In support of this view we found that excitation of the BPDE-DNA mixture is indistinguishable from the hydrocarbon, the length of the polydeoxynucleotide must be increased and the helix locally unwound (Waring, 1981). To measure unwinding of superhelical DNA by sedimentation techniques, experiments must be carried out at r values of ~0.1–0.2. Solubility limits the amount of BPDE that can be bound to DNA under the conditions used here to an r value of < 0.01. On the other hand, local unwinding as manifested by differences in superhelicities of covalently closed circular DNA can be measured electrophoretically at r values of ~0.002. We, therefore, carried out unwinding angle measurements by monitoring differences in superhelicity of SV40 DNA enzymatically relaxed with and without physically bound BPDE. The gel electrophoresis pattern obtained with various amounts of bound BPDE is presented in Fig. 3, while Fig. 4 demonstrates the linear relationship between BPDE concentration and the change in mean super helicity (Δr) of SV40 DNA. The gel pattern results in a Gaussian distribution of DNA topoisomers which differ from one another by unit values of superhelicity (Keller, 1975). The difference in r between control (Fig. 3, lanes A or H) and BPDE-treated DNA (lanes B–G) was obtained by measuring the distance between the average of the Gaussians and dividing it by the unit distance of super helicity. Thus, the difference in super helicity (Δr) between lanes H and G (Fig. 3) is 2.07. The unwinding angle of 13° obtained for the binding of BPDE to SV40 DNA utilizing the data from Fig. 4 is within the range of those reported for a number of established intercalating agents, i.e. 11–26° (Povirk et al., 1979; Quigley et al., 1980; Wright et al., 1980; Waring, 1981).

We also carried out the same type of unwinding angle analysis with the other hydrocarbon derivatives listed in Table I and the results of those analyses are presented in Table II. Relaxation of SV40 DNA in the presence of these compounds each yielded gel electrophoresis patterns (data not shown) similar to that obtained with BPDE. The unwinding angles for these hydrocarbons were remarkably similar, despite differences in the relaxation conditions which included alterations in temperature, concentration of hydrocarbon, levels of bound hydrocarbon, and salt concentrations.

The unwinding angle for binding of BPDE to SV40 DNA is the same in the presence of 50 and 200 mM NaCl, despite a 36% increase in the equilibrium constant in going from the higher to the lower salt conditions (see Table I). These results indicate that an insignificant amount of the hydrocarbon binds to the outside of the DNA helix. The validity of the unwinding angle measurements are supported by control values obtained with ethidium bromide. Using conditions identical with those for BPDE (with NaCl at 200 mM) to measure K(e), and SV40 DNA relaxation we obtained an unwinding angle for ethidium bromide.
bromide within experimental error of that reported in a number of studies (Wang, 1974; Pulleyblank and Morgan, 1975; Keller, 1975; Shure and Vinograd, 1976).

**Binding of BPDE to Denatured DNA**—The BPDE was mixed with denatured calf thymus DNA at pH 9.0 and an absorbance spectrum taken within 1 min of adding the hydrocarbon. Under these conditions the intercalation absorbance peak at 354 nm was reduced by about 80-85% compared to double-stranded DNA (Fig. 5). The spectrum of native DNA plus free BPDE are shown for comparison. These spectral results demonstrate that the BPDE does not physically bind to single-stranded DNA to the extent it does with duplex DNA.

**DISCUSSION**

Although the BPDE is unstable in protic solvents we were able to study its physical interaction with DNA by carrying out our experiments at an alkaline pH. Under these conditions the half-life of the hydrocarbon is of sufficient length to permit the measurements reported here prior to the accumulation of significant amounts of tetrols. It has been reported previously that the BPDE forms a reversible physical complex with DNA (Geacintov et al., 1981) and our studies confirm this observation. However, the question of what type of binding occurred was the major question of interest in this study. A direct investigation of the binding by x-ray crystallography is not possible due to the instability of the BPDE. Therefore, indirect evidence must be used to evaluate the principal mode(s) of binding. We have obtained several pieces of evidence which support the conclusion that BPDE and several of its derivatives physically bind to DNA by intercalation of the planar hydrocarbons between the stacked base pairs of the DNA duplex. This evidence can be summarized as follows: 1) the 10 nm shift in the absorbance spectrum of the hydrocarbons in the complex, 2) fluorescence quenching of the bound hydrocarbons, 3) salt dissociation of the complex, 4) binding of each of the hydrocarbons results in helix-unwinding angles near 13°, and 5) binding primarily occurs with native DNA.

An important question is whether the hydrocarbons bind significantly to the outside of the helix, in addition to intercalating into the DNA duplex. Tetrol has been reported, e.g. to form a nonintercalative complex with DNA as measured by equilibrium dialysis techniques (Ibanez et al., 1980). Again, in the case of the BPDE, this latter method is not practicable because of solvolysis. We have, however, several lines of evidence which lead to the conclusion that the BPDE binds principally by intercalation. Our experiments were carried out at low values of $r$. Proflavin is an example of an intercalating agent which binds to both the outside and inside of DNA, but appreciable binding in the former mode only occurs at high values of $r$ (Li and Crothers, 1969; Ramstein et al., 1980). Our binding studies were carried out at low concentrations of hydrocarbons, which obviate problems of aggregation. Aggregation could lead to ambiguous interpretation of the fluorescence evidence. The salt concentration was maintained at a moderate level. This decreases the likelihood of outside binding since this mode is more sensitive to salt than intercalation (Berman and Young, 1981). Finally, the unwinding angle exhibited by BPDE did not change with 50 and 200 mM NaCl. This latter piece of evidence is particularly strong support for the lack of significant outside binding, since the salt should have differential effects on the two binding modes. If the ratio of outside to inside binding were different in the presence of different concentrations of salt, then the calculated unwinding angle would be different under the two conditions.

The relative binding levels of the various hydrocarbons tested followed the order $BPDE > 7,8$-di(OH)H,BP $> 9,10$-di(OH),BP $> tetrot$. The predominant factor in the overall low binding of these hydrocarbons is due to their limited water solubility. The differences in binding within this series of hydrocarbons is probably related to solubility differences between the tetrot, on the one hand, and the monohydroxy and dihydroxy derivatives, on the other. The presence of cis-7,8-diols or trans-9,10-diols alone cannot account for the relative binding levels in this series of compounds. Alternatively, H-bonding characteristics may also contribute to these differences. Water-soluble and charged intercalators, e.g. proflavin (Berman and Young, 1981) bind to the outside of the DNA.

**Table II**

Unwinding angles of $BPDE$ and its derivatives

Superhelical SV40 DNA was completely relaxed with topoisomerase in the presence of several concentrations of each hydrocarbon. The extent of helix unwinding was determined by gel electrophoresis and this quantity used to calculate an unwinding angle due to the reversible binding of the hydrocarbon to DNA, as described under "Materials and Methods" and in the legends to Figs. 3 and 4.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relaxation time</th>
<th>Temperature</th>
<th>Relaxation Electro-</th>
<th>Unwinding angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>°C</td>
<td>Electrophoresis</td>
<td>degrees</td>
</tr>
<tr>
<td>BPDE</td>
<td>10</td>
<td>25</td>
<td>4</td>
<td>$13 \pm 1$</td>
</tr>
<tr>
<td>BPDE$^a$</td>
<td>10</td>
<td>25</td>
<td>4</td>
<td>$13 \pm 1$</td>
</tr>
<tr>
<td>7,8-di(OH)H,BP</td>
<td>30</td>
<td>8</td>
<td>37</td>
<td>$12 \pm 1$</td>
</tr>
<tr>
<td>9,10-di(OH)H,BP</td>
<td>30</td>
<td>8</td>
<td>37</td>
<td>$13 \pm 1$</td>
</tr>
<tr>
<td>7-(OH)H,BP</td>
<td>39</td>
<td>8</td>
<td>37</td>
<td>$13 \pm 2$</td>
</tr>
<tr>
<td>Tetrot</td>
<td>30</td>
<td>8</td>
<td>37</td>
<td>$13 \pm 3$</td>
</tr>
</tbody>
</table>

$^a$ Unwinding angle and $K_{on}$ were determined under the same conditions outlined above and in Table I but with 50 mM NaCl.
helix. In this case binding is probably driven, in part, by electrostatic interactions. Analogously, tetroleads may undergo binding to the outside of the helix due to H-bonding with functional groups. In the case of the diols and diol epoxides the predominant interaction is hydrophobic, which leads to intercalation of the planar hydrocarbons between the stacked bases of the duplex. This would not preclude H-bonding as a factor contributing to the conformation of the complexes, only that it is probably not dominant.

BPDE binds to single-stranded DNA at a level of about 10-20% that of double-stranded DNA. In contrast, proflavin binds even more strongly to denatured than to the helical form of DNA (Blake and Peacocke, 1968; Drummond et al., 1965). The complex, in the case of BPDE, could result from some form of nonintercalative binding or to intercalation into residual secondary structure still present in the denatured preparation. Some of our results can be interpreted in more than one way. However, the evidence taken together leads to the conclusion that physical binding of BPDE is the result of intercalation, and that under the conditions used here there is no significant outside bound form.

A number of reports on the structures of BPDE-DNA covalent complexes have appeared (Drinkwater et al., 1978; Geacintov et al., 1978; Hogan et al., 1981; Gamper et al., 1980). Drinkwater et al. (1978) and Gamper et al. (1980) have reported an unwinding angle of 26-30° for covalently bound BPDE to SV40 DNA. Why the physically bound BPDE unwinds the helix by half that reported for the covalent complex is unknown. There is little agreement on the mechanism of covalent adduct formation or on the conformation of the complex after the alkylation step. However, the formation of a reversible physical intercalation complex between BPDE and DNA has important consequences concerning the mechanism of covalent binding. We have measured the kinetics of physical intercalation by temperature-jump perturbation techniques and have found that the BPDE intercalates into DNA on a millisecond time scale. Under these same conditions the BPDE alkylates DNA on a time scale of minutes. Thus, intercalation occurs about 3 orders of magnitude faster than alkylation.

Previous studies (Meehan et al., 1977; Meehan and Straub, 1979) have demonstrated a number of specificities in the covalent binding of BPDE to DNA. The primary alkylation sites are exocyclic amino groups; guanine is the primary target among the bases, and the reaction is stereoselective. Based on these specificities, our preliminary kinetic data, and the results reported here we propose that physical intercalation is a prerequisite to covalent binding and that this mechanism would predominate at low values of $r$. In support of this scheme is the finding that many intercalating agents display a preferential physical binding to G-C-rich sequences (Müller and Gautier, 1975), a result which would explain the guanine alkylation specificity of BPDE. The sequence of events we propose is indicated in the following scheme:

\[
\text{DNA + BPDE} \overset{k_{1a}}{\rightarrow} \text{DNA-BPDE intercalation complex} \overset{k_{1b}}{\rightarrow} \text{DNA-BPDE covalent adduct}
\]

Most of the intercalated BPDE is hydrolyzed to tetrol (>98%) while only a minor fraction (1-2%) alkylates DNA. Conformational differences between the intercalation complexes as a result of binding to certain or specific base sequences may be responsible for the differential reactivities toward solvent and alkylation sites. Stereospecific alkylation could arise from kinetic or equilibrium differences in enantionmic intercalation or in the stereochemistry of the resultant physical complexes. Model building studies have suggested the feasibility of this type of mechanism. Elucidation of these mechanisms is important to an understanding of the chemical and biological properties of carcinogens.

Acknowledgments—We thank Dr. Kenneth Straub for the 9,10-di(OH)BP, BPDE, and 7,8-di(OH)BP, Dr. James Wang for the topoisomerase, Dr. John Hearst for support of Howard Gamper, and Denise Bond for assistance.

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