Structural Effects in Reactivity and Adduct Formation of Polycyclic Aromatic Epoxide and Diol Epoxide Derivatives with DNA: Comparison between 1-Oxiranylpyrene and Benzo[*a*]pyrenediol Epoxide[†]

Myung-Hoon Kim, Nicholas E. Geacintov,* Martin Pope, and Ronald G. Harvey

ABSTRACT: Reaction of 1-oxiranylpyrene (1-OP) with DNA and the structures of the covalent and noncovalent complexes formed were studied in aqueous media (5 mM phosphate buffer with 0.1 M NaCl, pH 7) by utilizing the techniques of absorption, fluorescence and linear dichroism spectroscopy in order to gain an understanding of possible structure–activity relationships for polycyclic aromatic hydrocarbon epoxides in tumorigenesis and carcinogenesis, and the results were compared with those obtained for the highly active benzo[*a*]pyrene diol epoxide (BaPDE). Like BaPDE, 1-OP undergoes acid-catalyzed hydrolysis with the pseudo-first-order rate constant $k = 4.6 \times 10^{-4}$ s^{-1} in the absence of DNA, which is about 10 times slower than in the case of BaPDE. In DNA solutions, this hydrolysis is catalyzed by a rapid formation of a physically bound complex of 1-OP···DNA, which subsequently undergoes either (1) hydrolysis to a diol derivative or (2) formation of a covalent adduct of 1-OP–DNA. The same value of the noncovalent binding constant ($K = 4000$ M^{-1}) is obtained for both 1-OP and for BaPDE, which suggests that the π-electron interaction between the pyrenyl moiety and the nucleic acid bases is the dominant factor in the formation of the physical complexes and that the two extra OH groups in BaPDE do not play a significant role in determining the value of the physical binding constant. The most striking difference between the structures of these two adducts is the linear dichroism (LD): unlike the BaPDE–DNA adduct which exhibits positive LD signals, characteristic of external binding sites, 1-OP–DNA adducts exhibit negative LD with the angle of orientation of the pyrenyl chromophore with respect to the DNA helix axis $\theta = 65 \pm 3°$, indicating conformations close to, but not identical with, those of dye–DNA intercalation complexes. The lower mutagenic activity of 1-OP relative to that of BaPDE may be related to these differences in the structures of the covalent DNA adducts.

It is well-known that polycyclic aromatic hydrocarbons can be metabolically activated in living cells to potent mutagens and carcinogens. For example, benzo[*a*]pyrene is enzymatically converted to a large number of oxygenated derivatives (Gelboin, 1980; Conney, 1982), including the diol epoxide BaPDE[1] (Harvey, 1981) which preferentially binds to the exocyclic amino group of guanine of DNA in vivo, in cultured mammalian cells, and in vitro (Weinstein et al., 1976; Gelboin, 1980; Meehan & Straub, 1979; Brookes & Osborne, 1983). The covalent binding of these reactive epoxide derivatives to DNA is widely believed to be an important critical event in the initiation stages of mutation and tumorigenesis (Singer & Grunberger, 1983).

The relationship between structural factors of the polycyclic molecules and their biological activity has long been a subject of great interest (Pullman, 1979; Dipple, 1976). For polycyclic aromatic molecules, the ultimate reactive forms are carbonium ions derived from the ring opening of epoxides (Dipple et al., 1968), and calculations of this type performed by Jerina & Lehr (1977) indicate that bay region epoxides and diol epoxides should be the most reactive metabolic derivatives of polycyclic aromatic hydrocarbons. Other structural effects such as absolute stereochemistry and the presence and absence of hydroxyl and alkyl groups, for example, also play an important role in determining the biological activities of these compounds (Harvey, 1981; Conney, 1982; Newbold et al., 1979).

In this work, the reactivity with DNA and the conformation of the DNA adducts formed are compared for 1-oxiranylpyrene (1-OP) and BaPDE. These compounds are structurally related, as shown in Figure 1, in that both molecules possess an epoxide group in the bay region, adjacent to the pyrene nucleus. Thus, according to the predictions of molecular orbital theory (Harvey, 1981), both compounds should be characterized by the same reactivity index, i.e., ease of formation of the reactive carbonium ion intermediate, which subsequently undergoes a covalent binding reaction with DNA. However, 1-OP lacks the carbon atoms at the 7,8-position of BaPDE, and of course, the hydroxyl groups attached to these carbon atoms. These structural differences may be important in determining the spatial conformations of DNA complexes, which are formed when these molecules bind to DNA, and may account for the differences in the biological activities of these compounds.

Some comparisons of the biological activities of 1-OP and BaPDE have been described in the literature. While BaPDE is a tumorigen on mouse skin (Levin et al., 1977), 1-OP is not (T. J. Slaga, private communication). Hsu et al. (1979) utilized a viral bioassay system in which the effect of alkylation of ϕX174 viral DNA by various epoxides and diol epoxides on the infectivity of *Escherichia coli* spheroplasts was determined. It was found that 1-OP was equally effective as BaPDE in producing total inhibition of viral replication.

[†] From the Chemistry Department and Radiation and Solid State Laboratory, New York University, New York, New York 10003 (M.-H.K., N.E.G., and M.P.), and the Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois 60637 (R.G.H.). *Received March 15, 1984.* This work was supported by the National Foundation for Cancer Research and in part by the Deparment of Energy [Contract E(11-11)2386] at the Radiation and Solid State Laboratory. We are grateful for equipment grants from the Camille and Henry Dreyfus Foundation, Inc., the National Science Foundation (Grant PCM-8108289) and the New York University Research Challenge Fund.

[1] Abbreviations: 1-OP, 1-oxiranylpyrene; BaPDE, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BaPE, 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; 1-OP–DNA, covalent complex of 1-OP and DNA; 1-OP···DNA, physical complex of 1-OP and DNA; BaPDE–DNA, covalent complex of BaPDE and DNA; BaPDE···DNA, physical complex of BaPDE and DNA; LD, linear dichroism.

0006-2960/84/0423-5433$01.50/0

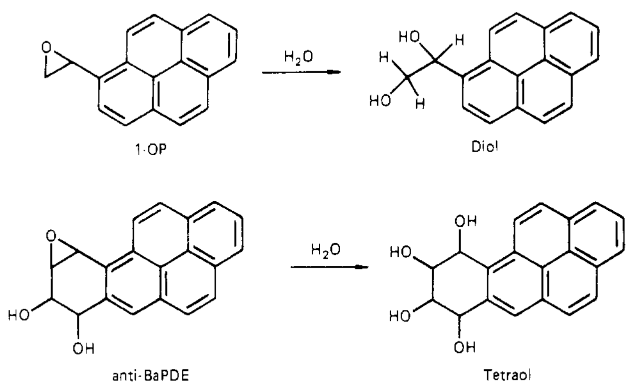

FIGURE 1: Structural formulas for 1-OP ($C_{18}H_{12}O$) and anti-BaPDE ($C_{20}H_{14}O$) and their hydrolysis products, the diol and tetraol derivatives.

However, a quantitative comparison of the mutagenicity of these two compounds in mammalian V-79 cell systems shows that 1-OP is about 4 times less mutagenic than BaPDE (Newbold et al., 1979; Brookes & Newbold, 1980). As a part of a general program on structure–reactivity studies, we have therefore investigated and compared the mechanisms of interaction of BaPDE and 1-OP with DNA in aqueous solutions under comparable, well-defined conditions utilizing a combination of spectroscopic techniques including linear dichroism, absorption, and fluorescence spectroscopy.

Materials and Methods

The racemic compounds 1-OP and BaPDE were synthesized at the University of Chicago according to methods published by Harvey et al. (1983) and Harvey & Fu (1978), respectively. The calf thymus DNA (Worthington Biochemicals, Freehold, NJ) was dissolved in 5 mM sodium phosphate buffer containing 0.1 M NaCl, the pH was adjusted to 7.05 ± 0.03, and the solutions were centrifuged at 10 000 rpm for 40 min before use. These samples of DNA were in the double-stranded form since, typically, the hyperchromicities determined by thermal denaturation methods were in the range of 39 ± 1%.

Preparation of Covalent DNA Adducts. The epoxides (concentrations 1–10 mM) were dissolved in tetrahydrofuran (THF). Small aliquots of these THF stock solutions were added to 10 mL of buffer solution containing DNA (1.2 mM in nucleotides), and the final concentration of epoxides in the reaction mixture ranged typically between 5 and 50 μM. The solubility of these compounds in buffer solution was determined by monitoring the validity of Beer's law as a function of epoxide concentration. In buffer solutions containing 0.2% THF, the solubility of BaPDE was found to be $\sim$10 μM, while that of 1-OP was only $\sim$1 μM. In some binding experiments in which 1-OP was utilized, an amount of 1-OP somewhat higher than the solubility of this compound was occasionally added to the DNA solution in order to obtain a maximal extent of product. Under these conditions fine microcrystals of 1-OP were formed (easily visible by means of a small ultraviolet lamp) just after mixing, but these microcrystals gradually disappeared as the reaction was allowed to proceed. The mixtures were incubated at 37 °C for 4 h. The reaction mixtures were extracted with ether (12 times) and precipitated with cold ethanol in the presence of 0.1 M NaCl to remove all of the noncovalently bound diols, or tetraols, derived from the hydrolysis of 1-OP and BaPDE, respectively. Another method for extracting the hydrolyzed and physically bound hydrolysis products was also utilized; this method involved the separation of the modified DNA from the reaction mixture by using hydroxyapatite column chromatography (Bio-Rad, Richmond, CA) according to the technique described by Beland et al. (1979) and Adriaenssens et al. (1982). Both extraction methods gave the same results. The extent of covalent binding was estimated spectroscopically by utilizing a Perkin-Elmer UV 320 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT).

In determining the molar concentration of BaPDE in the aqueous buffer solutions, we utilized the value of 29 000 ± 1000 at 343 nm determined by us previously (Geacintov et al., 1983), which is the same value as given by Weinstein et al. (1976) for BaPDE bound covalently to nucleic acids. The molar absorptivity of 1-OP in the aqueous solutions was determined by comparing the absorbances as a function of 1-OP concentration in the buffer solutions and in methyl alcohol. For this latter solvent the molar extinction coefficient reported at the absorption maximum at 342 nm was 48 900 M^{-1} cm^{-1} (Yang et al., 1978), but in the aqueous buffer solutions we found that this value is only 27 000 ± 2000 M^{-1} cm^{-1}; the relatively high error bars in this value arise because of the low solubility of 1-OP in aqueous media.

Fluorescence Spectrophotometry. The kinetics of the degradation of 1-OP and BaPDE in aqueous DNA solution were measured as described previously (Geacintov et al., 1982a); we found that, just as in the case of BaPDE, the diol derived from the hydrolysis of 1-OP is fluorescent, while the epoxide either is nonfluorescent or is characterized by a low fluorescence yield. As 1-OP is converted to the diol, the fluorescence intensity of a typical aqueous reaction mixture increases with time according to the first-order rate law:

$$F(t) = F_{\max}(1 - e^{-k_H t}) \qquad (1)$$

where $F(t)$ and $F_{\max}$ are the fluorescence intensities at time t and $t \to \infty$, respectively, and k_H is the pseudo-first-order rate constant characterizing the disappearance of the epoxides.

In determining the fluorescence kinetics, as well as the fluorescence excitation and emission spectra, a Hitachi MPF-2A fluorescence spectrophotometer (Perkin-Elmer Corp., Norwalk, CT), or a photon counting spectrophotometer (SPEX Model 1902 fluorolog, SPEX Industries, Metuchen, NJ), was utilized. In all of the fluorescence kinetic experiments the fluorescence excitation wavelength was 343 ± 3 nm, while the viewing wavelength was 400 ± 5 nm.

Linear Dichroism. In these measurements the DNA molecules are oriented either by an applied electric field (Frederick & Houssier, 1973) or by flow (Wada & Kozawa, 1964), and the dichroism in the region of the absorption bands of the pyrenyl chromophore is probed by utilizing polarized light. The linear dichroism ΔA is defined as

$$\Delta A = A_{\parallel} - A_{\perp} \qquad (2)$$

where $A_{\parallel}$ and $A_{\perp}$ are the absorbances of the solution measured with the polarizer oriented parallel and perpendicular to the direction of orientation (the axis of the DNA helix). The details of the electric field induced linear dichroism are fully described elsewhere (Geacintov et al., 1978). In the flow orientation method the DNA solutions are placed within a concentric gap (0.7 mm wide) formed by an outer stationary quartz cylinder and an inner rotating cylinder, 29 mm in diameter. This apparatus is similar to the one described by Wada & Kozawa (1964), and the maximum degree of orientation of the DNA molecules is obtained at speeds of rotation of about 1000 rpm (Wada & Kozawa, 1964).

Results

Absorption and Fluorescence Properties of 1-OP–DNA Complexes. The free epoxide in the aqueous buffer solution

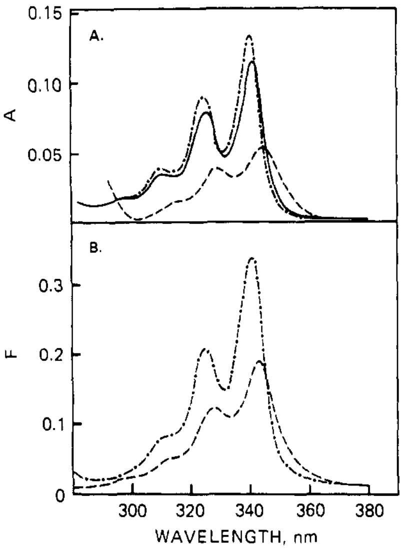

FIGURE 2: (A) Absorption (*A*) and (B) fluorescence excitation (*F*) spectra: (—) free 1-OP (5×10^{-6} M), (···) hydrolysis product of 1-OP (diol derivative), and (- - -) covalent complex of 1-OP–DNA. The fluorescence intensity is in arbitrary units, and the emission was monitored at 398 nm.

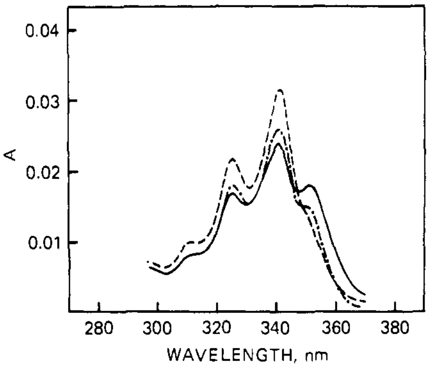

FIGURE 3: Absorption spectra of a 1-OP–DNA reaction mixture as a function of time at pH 7.05: (—) 15 s, (···) 3 min, and (- - -) 30 min after adding 1-OP to the DNA solution. [1-OP] = 2×10^{-6} M and [DNA] = 6.2×10^{-4} M.

exhibits absorption maxima at 311, 326, and 342 nm, while the hydrolysis product of 1-OP, the diol (Figure 1), exhibits a slightly blue-shifted (by about 1 nm) absorption spectrum (Figure 2). In the presence of DNA a new band develops at 352 nm (Figure 3). Analogous results are observed with BaPDE under similar conditions (Geacintov et al., 1980); this new red-shifted band is attributed to intercalative complex formation. With increasing time the epoxide is gradually converted to the diol, and the intensity of this band decreases and is finally reduced to a shoulder which is attributed to the physical binding of the diol to DNA. The peak at 341 nm increases in height as the absorbance at 352 nm decreases with time, indicating that the diols are bound less strongly to DNA by the intercalation mechanism than the epoxide. Again, this behavior parallels the observations obtained with BaPDE (Geacintov et al., 1980; Meehan et al., 1982).

Upon extraction of the noncovalently bound diols, the absorption maxima of the covalently bound pyrenyl chromophore at 310, 330, and 345 nm become apparent (Figure 2A). This represents a red shift of 3–4 nm as compared to the absorption maximum of 1-OP in aqueous buffer solution. In the case of BaPDE the corresponding red shift upon covalent binding of the diol epoxide to DNA is only 1–2 nm.

The fluorescence excitation spectra of the free diol and the covalent adduct are compared in Figure 2B. A red shift of about 3 nm is also noted for covalent adducts.

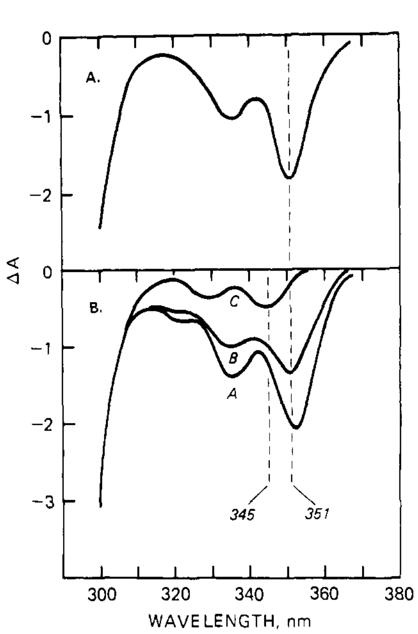

FIGURE 4: Linear dichroism spectra (ΔA). A. Mixture of the diol and DNA. B. (A) Mixture of 1-OP and DNA immediately after mixing. (B) Mixture of 1-OP and DNA at the end of reaction (30 min after mixing). (C) Covalent adduct of 1-OP–DNA. Concentration of pyrenyl chromophores = 2×10^{-6} M and that of DNA = 1.24×10^{-3} M for (A) and (B).

Linear Dichroism (*LD*). In these measurements the average orientation of the long axis within the plane of the pyrenyl chromophore relative to the average orientation of the DNA bases is determined (Geacintov et al., 1978).

In Figure 3, it is shown that a new absorption maximum develops at 352 nm when 1-OP is added to an aqueous DNA solution. Since, 15 s after mixing, the extent of covalent binding and hydrolysis is still negligible, this 352-nm maximum is attributed to the formation of a noncovalent 1-OP···DNA complex. This conclusion is confirmed by the linear dichroism spectrum of such a solution, taken within 25–45 s of mixing and shown in Figure 4 (a complete wavelength scan requires about 20 s). Noting that only those molecules which are bound to the oriented DNA molecules contribute to the linear dichroism spectrum, the initial ΔA spectrum reflects the absorption spectrum of the bound chromophore, in this case, physically complexed 1-OP (curve A in Figure 4B). With increasing time the magnitude of the ΔA spectrum decreases and finally reaches a steady-state value after 15 min (curve B in Figure 4B). This decrease is due to the gradual hydrolysis of 1-OP to the diol, and the accompanying changes in the absorption spectra are shown in Figure 3.

The linear dichroism spectrum of the diol added to DNA is shown in Figure 4A. The linear dichroism spectra resemble the inverted absorption spectra, as expected. In the case of the ΔA spectrum of the physically bound diol (Figure 4A), the ΔA minima occur at 334 and 351 nm. The equilibrated ΔA spectrum of the solution to which 1-OP was added originally (curve B in Figure 4B) resembles the ΔA spectrum of the diol···DNA physical complexes.

Upon extraction of the equilibrated 1-OP···DNA reaction mixture with ether, the noncovalently bound diols are removed and a smaller, blue-shifted ΔA spectrum due to covalently bound residues is obtained (curve C, Figure 4B). This linear dichroism spectrum is negative, in contrast to the positive ΔA spectrum obtained in the case of BaPDE–DNA covalent adducts (Geacintov et al., 1978). The minima at 330 and 345 nm are closely correlated with the absorption spectra of the

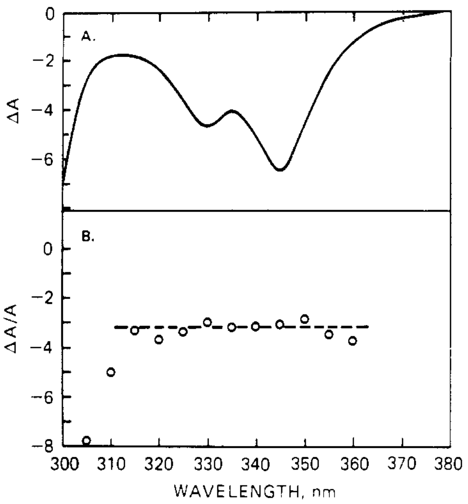

FIGURE 5: (A) Linear dichroism spectra (Δ*A*) of 1-OP–DNA covalent complex. (B) Reduced linear dichroism (Δ*A*/*A*) for the same complex. Both Δ*A* and Δ*A*/*A* are in arbitrary units.

Table I: Summary of Linear Dichroism Results

adduct	$(\Delta A/A)_\lambda/(\Delta A/A)_{260}$	λ (nm)	θ^a (deg)
covalent 1-OP–DNA	0.48 ± 0.06	345	65 ± 3
covalent BaPE–DNA[b]	0.57 ± 0.10	354	68 ± 4
covalent BaPDE–DNA[c]	−1.0	340	<35
noncovalent 1-OP···DNA	0.64 ± 0.06	351	70 ± 2

[a] Average angle between long axis of pyrenyl residues and the axis of the DNA helix. [b] From Geacintov et al. (1982b). [c] From Geacintov et al. (1978).

covalent complexes (Figure 2A).

Approximate average orientation angles θ of the long, in-plane axis of the pyrenyl chromophore with respect to the axis of the DNA helix can be estimated by examining the reduced linear dichroism $\Delta A/A$, which is related to the orientation angle θ according to the equation (Frederick & Houssier, 1973)

$$\Delta A/A \propto 3\cos^2\theta - 1 \qquad (3)$$

Plots of typical ΔA and $\Delta A/A$ spectra for a covalent 1-OP–DNA adduct are shown in Figure 5. The sharp decrease below 315 nm is due to the DNA bases, but above 315 nm the values of $\Delta A/A$ are constant as a function of wavelength within experimental error. A constant value of $\Delta A/A$ is indicative of a single orientation angle θ (or a small range of orientation angles). When the $\Delta A/A$ values at 345 nm are compared to the values of $\Delta A/A$ for the DNA bases at 260 nm (Geacintov et al., 1978; Houssier, 1981), orientation angles of $\theta = 65 \pm 3°$ are obtained (Table I). In the case of classical intercalation acridine dye–DNA complexes, an angle closer to 90° is expected (Houssier, 1981; Ramstein et al., 1973; Geacintov et al., 1978). A classical intercalative geometry of the type observed with acridine dyes bound noncovalently to DNA can thus be also ruled out for the covalent adducts on the basis of these linear dichroism results.

Results of analogous calculations of the orientation angles of the noncovalent 1-OP complex just after mixing the solutions are also given in Table I. Angles of 70 ± 2° are obtained which are somewhat larger than those obtained for the covalent adduct. Thus, there is a small apparent change in the orientation of the long axis of the pyrene residue upon covalent bond formation.

Acceleration of Hydrolysis of 1-OP by DNA. The rate of hydrolysis of 1-OP increases with increasing DNA concentration, and this phenomenon is very similar to the one observed for BaPDE (Geacintov et al., 1980, 1982a; Kootstra et al., 1980; Macleod et al., 1982). The time course of the increase in the fluorescence at different concentrations of DNA is shown in Figure 6. The pseudo-first-order hydrolysis rate constant k_H can be obtained by a computer fit of eq 1 to the data. Just as in the case of BaPDE (Geacintov et al., 1980, 1982a; Kootstra et al., 1980), the maximal fluorescence intensity F_{max} decreases with increasing DNA concentration (not shown), which is attributed to the formation of intercalative, nonfluorescent diol···DNA complexes.

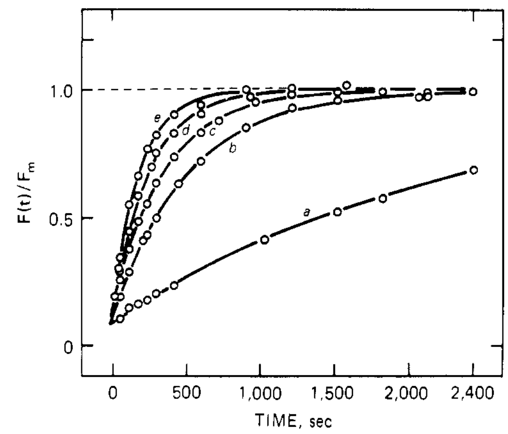

FIGURE 6: Effect of DNA concentration on the hydrolysis rate of 1-OP. Normalized fluorescence intensity $[F(t)/F_{max}]$ vs. time. Concentration of DNA: (a) 0.00×10^{-3}, (b) 0.078×10^{-3}, (c) 0.155×10^{-3}, (d) 0.621×10^{-3}, (e) 3.11×10^{-3} M. The solid lines (—) are plots of the first-order rate equation (eq 1), and circles (○) represent the experimental values.

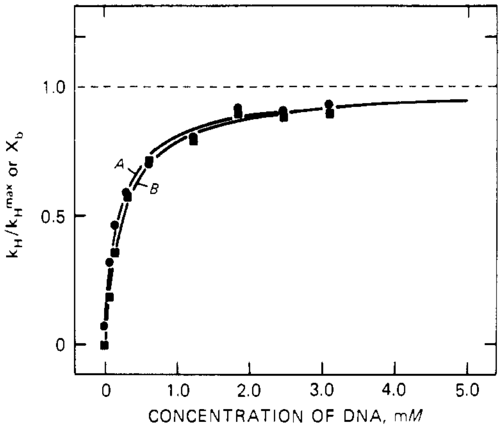

FIGURE 7: (A) Dependence of the normalized hydrolysis rate (k_H/k_H^{max}) (epoxide → diol) on the concentration of DNA. The solid line (A) is a plot of eq 5 with values of $K' = 4090$ and $k_3 = 5.81 \times 10^{-3}$ s^{-1} as determined by fitting the experimental points (●) with a nonlinear least-squares computer program. (B) Dependence of fraction of epoxide molecules (X_b) intercalated into DNA as determined from the absorbances at 352 nm shortly after mixing. The solid line (B) is a plot of eq 6 with values of $K = 3900$ as determined by fitting the experimental points (■) with the computer program.

The dependence of k_H on the DNA concentration is depicted in Figure 7. At concentrations of DNA above 10^{-3} M, the value of k_H approaches a limiting constant value.

This behavior may be understood in terms of a simple kinetic scheme involving the formation of a noncovalent 1-OP···DNA complex, followed by hydrolysis of 1-OP at the DNA binding sites (Geacintov et al., 1982a).

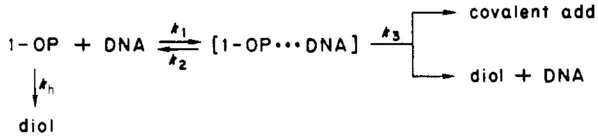
$$\begin{array}{l} \text{1-OP} + \text{DNA} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [\text{1-OP}\cdots\text{DNA}] \xrightarrow{k_3} \begin{cases} \text{covalent adducts} \\ \text{diol} + \text{DNA} \end{cases} \\ \quad \downarrow k_h \\ \text{diol} \end{array} \qquad (4)$$

Table II: Comparison of Reaction Rate Constants (k_h, k_3) and Physical Binding Constants (K) for 1-OP and BaPDE[a]

	1-OP	BaPDE
K (M^{-1})	4000 ± 300	4100 ± 400
k_h (s^{-1}) (free)	$(0.46 \pm 0.06) \times 10^{-3}$	$(3.4 \pm 0.3) \times 10^{-3}$
k_3 (s^{-1}) (DNA complex)	$(5.8 \pm 0.1) \times 10^{-3}$	$(15 \pm 1) \times 10^{-3}$
covalent binding[b] (%)	8 ± 1	8 ± 1

[a] All measurements are performed in 5 mM phosphate buffer (pH 7) in the presence of 0.1 M NaCl. [b] Fraction of molecules that undergo reaction by covalent binding to DNA rather than hydrolysis to diols, or tetraols.

According to this model, the overall hydrolysis constant, k_H, depends on the DNA concentration by the following expression:

$$k_H = \frac{k_h}{1 + K[\mathrm{DNA}]} + \frac{k_3 K[\mathrm{DNA}]}{1 + K[\mathrm{DNA}]} \qquad (5)$$

in which K is approximately equal to the association constant for the formation of physical (noncovalent) 1-OP···DNA complexes, k_3 is the rate constant of reaction of 1-OP at these physical binding sites, and k_h is the hydrolysis rate constant of 1-OP in the buffer solution.

The equilibrium constant of the epoxide, K, can be estimated by monitoring the absorption maximum at 352 nm due to the intercalated and noncovalently bound 1-OP molecules immediately after mixing the 1-OP into the DNA solutions. The behavior of the absorbance monitored at 352 nm in normalized units as a function of the DNA concentration is also shown in Figure 7. Within experimental error, the DNA concentration dependence of k_H and of the fraction X_b of 1-OP molecules, which are bound intercalatively to DNA, coincides as predicted by eq 5 for small values of k_h, since X_b is given by

$$X_b = \frac{K[\mathrm{DNA}]}{1 + K[\mathrm{DNA}]} \qquad (6)$$

A comparison of values of k_h, k_3, and K obtained for 1-OP and BaPDE under identical conditions is given in Table II.

Analysis of Products. Preliminary analysis of the hydrolysis products obtained 24 h after mixing reveals the following: (a) In the buffer alone, about 90% of the epoxides are converted to diols, with a phosphate derivative as a minor product (~10%). Although a carbonyl derivative is also observed as a result of the opening of the epoxide ring at the beginning of the reaction, this product disappears completely after 24 h. (b) In the presence of DNA (1.2 mM) in the buffer, about 90% of the epoxides are converted to diols, and about 8% form covalent adducts with DNA (Table II). A trace amount of aldehyde (<1%) is also found, and the phosphate derivative of the epoxide is not observed in the ether extracts. Further details of the product analysis by HPLC and mass spectrometry will be published elsewhere.

Discussion

Physical Complex Formation. The behavior of 1-OP and that of BaPDE are analogous; noncovalent complexes with DNA, characterized by relatively large (~10 nm) red shifts in the absorption spectra, are formed immediately upon mixing. The binding constants K obtained under identical conditions of ionic strength and composition of buffer solutions are the same for both molecules, within experimental error (Table II). The value of $K = 4000$ M^{-1} is lower than the one reported for BaPDE previously [~12 000 M^{-1} for BaPDE (Geacintov et al., 1982a)], but this difference may be explained in terms of differences in the ionic strengths (Michaud et al., 1983). In this work the NaCl concentration was 0.1 M, thus accounting for the lower values of K. Since the physical binding constants are the same for 1-OP and BaPDE, the formation of noncovalent complexes appears to be neither hindered nor enhanced by the presence of the 7,8-carbon atoms in BaPDE and the hydroxyl groups attached to these atoms. The physical interaction leading to physical complex formation thus seems to be contributed mostly by the pyrenyl moiety. The 10-nm red shifts in the absorption spectra (Mantione, 1973) and the negative LD spectra are consistent with an intercalative mechanism of bindings of the physical 1-OP···DNA complex, although the orientation angle of $\theta \approx 70°$ (Table I) is smaller than the value of 90° expected for ideal intercalation complexes.

Rate of Reaction of Epoxides. The hydrolysis rate constant k_h of 1-OP in buffer solution (without DNA) is almost 10 times smaller than k_h for BaPDE (Table II). While simple molecular orbital theory predicts that the ease of carbonium ion formation should be the same for both molecules (Hsu et al., 1979), this result confirms that other structural factors also play an important role in the hydrolysis rates of epoxides (Jerina et al., 1980). This difference in reactivities may be rationalized in terms of the following factors: (1) The benzylic carbon–oxygen bond in the epoxide ring of BaPDE is better aligned than in the case of 1-OP to provide better overlap between the developing π-orbital of the carbonium ion in the transition state and the aromatic π-electron ring system (Whalen et al., 1978, 1979; Sayer et al., 1982); because the epoxide ring in 1-OP is free to rotate with respect to the pyrenyl ring, thus hindering a favorable alignment of the π-orbitals during the formation of carbonium ions, the value of k_h is lower for 1-OP. (2) The larger value of k_h in the case of BaPDE may also be due to the destabilization of the π-electron energy levels. According to photoelectron studies and molecular orbital calculation by Akiyama et al. (1979), all of the highest occupied π-orbitals in BaPDE are destabilized as compared to those in pyrene and in 1-OP. Such destabilization of the π-system in BaPDE is expected to enhance the ability of the diol epoxide to form intermediates that possess either a full or a partial positive charge on the saturated benzylic ring system of BaPDE (Akiyama et al., 1979), thus also accounting for the higher k_h value in BaPDE.

The value of k_3, under identical conditions, is about 13 and 4 times higher, respectively, than k_h for 1-OP and BaPDE, respectively. While the exact reasons for this enhancement in the reactivities upon complexation to DNA are presently not understood (Geacintov et al., 1982a; Michaud et al., 1983), the reactions are specific and general acid catalyzed and are likely to depend on the stereochemistry of these noncovalent complexes. The factors which may affect differences in k_3 for 1-OP and BaPDE might be (1) hydrogen bonding involving the hydroxyl groups of BaPDE and DNA sites which may contribute to a favorable orientation of the reaction intermediate and (2) free rotation of the oxiranyl group allowing adoption of an energetically preferred orientation in which ring opening is not favored (less orbital overlap) and nucleophilic attack by water is hindered. The additional reaction pathway of covalent binding which can occur in the DNA complexes and thus affect the values of k_3 cannot account for the differences in k_3/k_h for 1-OP and BaPDE since only about 8% of the molecules initially present react with DNA covalently in both cases.

Conformation of Covalent Adducts. While there are no apparent differences in the properties of the physical DNA complexes derived from 1-OP and BaPDE, there are striking differences in the conformations of the covalent adducts. The linear dichroism ΔA is negative for both types of physical complexes (not shown here for BaPDE), but the ΔA spectrum of the covalent adducts is positive in the case of BaPDE (Geacintov et al., 1978) and is negative in the case of 1-OP adducts (Figure 5). In the BaPDE case, the orientation angle θ is less than 35°, while for 1-OP adducts θ equals 65 ± 3°. Thus, there is a much more significant change in the orientation of the pyrenyl chromophore upon covalent binding in the case of BaPDE than in the case of 1-OP.

The large differences in the conformations of the 1-OP and BaPDE covalent adducts indicate that the hydroxyl groups at the 7- and 8-positions of BaPDE play a crucial role in the stereochemistry of covalent binding. The covalent adduct derived from the binding of 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BaPE), the analogue of BaPDE without the OH groups at the 7- and 8-positions which is also less mutagenic than BaPDE (Conney, 1982), has the same conformation ($\theta \approx 68°$; Table I) as the adduct derived from 1-OP (Geacintov et al., 1982b), indicating that the presence and configuration of the hydroxyl groups, rather than the 7,8-carbon atoms, are crucial in determining the conformations of the covalent complexes. The hydroxyl groups in BaPDE provide additional opportunities for noncovalent interactions with DNA, possibly by hydrogen bonding of either the phosphate groups or nucleophilic groups in the DNA bases. Alternatively these OH groups may determine the geometry of the reactive complexes for simple steric reasons.

Conclusions

A detailed comparison of the reaction pathways of 1-OP and BaPDE in aqueous DNA solutions indicates that intercalative physical complexes with similar binding constants are formed in both cases. While the reaction rate of DNA-bound 1-OP (mostly hydrolysis to diols) is about 2.5 times slower than that of BaPDE, the fraction of molecules that undergo covalent binding to DNA, ~8%, is the same within experimental error in both cases. Striking differences, however, are found in the conformations of the covalent adducts. There is a remarkable reorientation of the pyrenyl chromophore as the intercalated, physically complexed BaPDE molecule is converted to an external covalently bound form. In the case of 1-OP, the apparent orientations of the physical and covalently bound forms are not markedly different, even though the pyrenyl chromophore does not display the characteristics of ideal intercalative conformations.

These results suggest that the known lower mutagenic and tumorigenic activity of 1-OP as compared to that of BaPDE may be related to differences in the structure of the covalent DNA adducts and possibly to differences in reactivity with DNA, rather than to the extent of reaction. It may be that the external location of the pyrenyl chromophore in covalent BaPDE–DNA adducts is of particular significance in determining the enhanced biological activity of BaPDE as compared to the activities of the structurally similar 1-OP and BaPE molecules.

Acknowledgments

We are grateful to Dr. Y. Mnyukh for measuring the linear dichroism spectra and to Dr. V. Ibanez for helpful discussions on several experimental aspects.

Registry No. 1-OP, 92078-74-5; BaPDE, 55097-80-8; 1-OP diol derivative, 92078-75-6; BaPDE tetraol derivative, 59957-91-4.

References

Adriaenssens, P. I., Bixler, C. T., & Anderson, M. W. (1982) *Anal. Biochem. 123*, 162–169.

Akiyama, I., Li, R. C., LeBreton, P. R., Fu, P. P., & Harvey, R. G. (1979) *J. Phys. Chem. 83*, 2887–3003.

Beland, F. A., Dooley, K. L., & Casciano, D. A. (1979) *J. Chromatogr. 174*, 177–186.

Brookes, P., & Newbold, R. F. (1980) in *Carcinogenesis: Fundamental Mechanisms and Environmental Effects* (Pullman, B., Ts'o, P. O. P., & Gelboin, H., Eds.) pp 81–90, D. Reidel Publishing Co., Dordrecht, The Netherlands.

Brookes, P., & Osborne, M. R. (1983) *Carcinogenesis* (*London*) *3*, 1223–1226.

Conney, A. H. (1982) *Cancer Res. 42*, 4875–4917.

Dipple, A. (1976) in *Chemical Carcinogens* (Searle, C. E., Ed.) ACS Monogr. No. 193, pp 245–314, American Chemical Society, Washington, DC.

Dipple, A., Lawley, P. D., & Brookes, P. (1968) *Eur. J. Cancer 4*, 439–463.

Frederic, R., & Houssier, C. (1973) *Electric Dichroism and Electric Birefringence*, Clarendon Press, Oxford.

Geacintov, N. E., Gagliano, A., Ivanovic, V., & Weinstein, I. B. (1978) *Biochemistry 17*, 5256–5262.

Geacintov, N. E., Ibanez, V., Gagliano, A. G., Yoshida, H., & Harvey, R. G. (1980) *Biochem. Biophys. Res. Commun. 92*, 1335–1342.

Geacintov, N. E., Yoshida, H., Ibanez, V., & Harvey, R. G. (1982a) *Biochemistry 21*, 1864–1869.

Geacintov, N. E., Gagliano, A. G., Ibanez, V., & Harvey, R. G. (1982b) *Carcinogenesis* (*London*) *3*, 247–253.

Geacintov, N. E., Ibanez, V., Benjamin, M. J., Hibshoosh, H., & Harvey, R. G. (1983) in *Polynuclear Aromatic Hydrocarbon: Formation, Metabolism and Measurement* (Cooke, M., & Dennis, A. J., Eds.) Battelle Press, Columbus, OH, pp 559–570.

Gelboin, H. V. (1980) *Physiol. Rev. 60*, 1107–1166.

Harvey, R. G. (1981) *Acc. Chem. Res. 14*, 218–226.

Harvey, R. G., & Fu, P. P. (1978) in *Polycyclic Hydrocarbons and Cancer* (Gelboin, H. V., & T'so, P. O. P., Eds.) Vol. 1, pp 133–165, Academic Press, New York.

Harvey, R. G., Konieczny, M., & Pataki, J. (1983) *J. Org. Chem. 48*, 2930–2932.

Houssier, C. (1981) in *Molecular Electro-Optics* (Krause, S., Ed.) pp 363–398, Plenum Press, New York.

Hsu, W. T., Sagher, D., Lin, F. J., Harvey, R. G., Fu, P. P., & Weiss, S. B., (1979) *Biochem. Biophys. Res. Commun. 87*, 416–423.

Jerina, D. M., & Lehr, R. G. (1977) in *Microsomes and Drug Oxidation* (Ullrich, N., Roots, I., Hildebrandt, A., Estabrook, R. E., Conney, A. H., Eds.) p 709, Pergamon Press, New York.

Jerina, D. M., Sayer, J. M., Thakker, D. R., & Yagi, H. (1980) in *Carcinogenesis: Fundamental Mechanisms and Environmental Effects* (Pullman, B., Ts'o, P. O. P., and Gelboin, H., Eds.) pp 1–12, D. Reidel Publishing Co., Dordrecht, The Netherlands.

Kootstra, A., Haas, B. L., & Slaga, T. J. (1980) *Biochem. Biophys. Res. Commun. 94*, 1432–1438.

Levin, W., Wood, A. W., Wislocki, P. G., Kapitulnik, J., Yagi, H., Jerina, D. M., & Conney, A. H. (1977) *Cancer Res. 37*, 3356–3361.

MacLeod, M. C., & Selkirk, J. K. (1982) *Carcinogenesis* (*London*) *3*, 287–292.

Mantione, M. J. (1973) *Photochem. Photobiol. 17*, 169–179.

Meehan, T., & Straub, K. (1979) *Nature* (*London*) *277*, 410–412.

Meehan, T., Gamper, H., & Becker, J. F. (1982) *J. Biol. Chem. 257*, 10479–10485.

Biochemistry **1984**, *23*, 5439–5446

Michaud, D. P., Gupta, S. G, Whalen, D. L., Sayer, J. M., & Jerina, M. (1983) *Chem.-Biol. Interact. 44*, 41–51.

Newbold, R. P., Brookes, P., & Harvey, R. G. (1979) *Int. J. Cancer 24*, 203–209.

Pullman, B. (1979) *Int. J. Quant. Chem. 16*, 669–689.

Ramstein, J., Houssier, C., & Levey, M. (1973) *Biochim. Biophys. Acta 335*, 54–68.

Sayer, J. M., Yagi, H., Silverton, J. V., Feldman, S. L., Whalen, D. L., & Jerina, D. M. (1982) *J. Am. Chem. Soc. 104*, 1972–1978.

Singer, B., & Grunberger, D. (1983) *Molecular Biology of Mutagens and Carcinogens*, Plenum Press, New York.

Wada, A., & Kozawa, S. (1964) *J. Polym. Sci. 2*, 853–864.

Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H., & Nakanishi, K. (1976) *Science* (*Washington, D.C.*) *193*, 592–595.

Whalen, D. L., Ross, A. M., Yagi, H., Karle, J. M., & Jerina, D. M. (1978) *J. Am. Chem. Soc. 100*, 5218–5221.

Whalen, D. L., Ross, A. M. Montemarano, J. A., Thakker, D. R., Yagi, H., Jerina, D. M. (1979) *J. Am. Chem. Soc. 101*, 5086–5088.

Yang, N. C., Chiang, W., Leonov, D., Bilyk, I., & Kim, B. (1978) *J. Org. Chem. 43*, 3425–3427.

Comparison of the Conformation of Poly(dI-dC) with Poly(dI-dbr^{5}C) and the B and Z Forms of Poly(dG-dC). One- and Two-Dimensional NMR Studies[†]

Peter A. Mirau and David R. Kearns*

ABSTRACT: One- and two-dimensional nuclear Overhauser effects (2D NOE) have been used to compare the conformational properties of 60–80 base pair long duplexes of the synthetic DNA polymer poly(dI-dC) with those of poly(dI-dbr^{5}C) and poly(dG-dC) in the B and Z conformations. Cross peaks in the 2D NOE spectra arising from proton–proton dipolar interactions which are more or less independent of the DNA conformation are used to assign the spectra of these molecules. Other cross peaks are sensitive to the conformational details, and these are used to make deductions about the average conformation in solution. The proton–proton interactions that give rise to the cross peaks in the 2D NOE spectrum of poly(dI-dC) are indicative of a B family conformation and rule out the possibility of some alternative conformations, including A, Z, alternating B, and left-handed B-DNA. The spectra are similar to those obtained from B-form poly(dI-dbr^{5}C) and poly(dG-dC) but different from Z-form poly(dG-dC). Taken together, these results indicate that the solution conformation of poly(dI-dC) is not unusual but more closely resembles that of other B-form DNAs.

The sequence-dependent properties of DNA play a key role in the expression of information contained in DNA sequences (Wells et al., 1980), and the determination of the physical basis for the recognition of specific sequences is one of the fundamental unsolved problems in molecular biology. Among DNA features that might be recognized by proteins and drugs are differences in conformation, chemical structure, topology, and dynamics. The recent discovery of Z-DNA dramatically demonstrates that DNA may exist in a variety of conformational states that depend on the solvent conditions and the duplex sequence (Pohl & Jovin, 1972; Wang et al., 1979). Although a variety of techniques have been used to demonstrate that poly(dG-dC) adopts a left-handed helix under the appropriate conditions (Pohl & Jovin, 1972; Nickol et al., 1982; Thamann et al., 1981; van de Sande & Jovin, 1982), the situation is much less clear regarding the probable conformational states of other synthetic alternating purine–pyrimidine DNAs. It has been observed, for example, that under the appropriate solvent conditions the conformations of poly(dA-dT) and poly(dA-dC)·poly(dG-dT) are spectroscopically different from B-form DNA (Zimmer et al., 1982).

The conformation of the alternating DNA duplex poly(dI-dC) has long been the subject of controversy. In solution, the long wavelength band in the circular dichroic (CD) spectrum of poly(dI-dC) is inverted compared to that of normal B-form DNA, and a "bizarre" X-ray diffraction pattern for the sodium salt of the fibers at 75% relative humidity has been reported (Mitsui et al., 1970; Grant et al., 1972). Such data have been interpreted in terms of a left-handed helix by one group (Mitsui et al., 1970) and a right-handed D-form helix by another (Ramaswamy et al., 1982). More recently the data have been interpreted in terms of a left-handed double-helical structure in which the bases retain their usual anti orientation about the glycosidic bond but form Hoogsteen-type base pairs (Drew & Dickerson, 1982) (Figure 1). In contrast with the unusual structures which have been proposed to account for the X-ray diffraction data, some spectroscopic and enzymatic studies suggest that the conformation of poly(dI-dC) resembles "normal" B-DNA. DNA polymerase, ligase, and nucleases utilize poly(dI-dC) as well as other synthetic DNA polymers (Grant et al., 1972), and the ^{31}P spectrum of poly(dI-dC) is similar to that of poly(dA-dT) (Cohen et al., 1981). In addition, the vacuum UV CD spectrum of poly(dI-dC) resembles other B-form DNA polymers (Sutherland & Griffin, 1983).

In this study we have used one- and two-dimensional (2D) nuclear Overhauser effects (NOE) to examine the solution conformation of poly(dI-dC). These studies are easily performed on 60–80 base pair long fragments, and we report the comparison of the conformational features of poly(dI-dC) with

---

[†] From the Department of Chemistry, University of California, San Diego, La Jolla, California 92093. *Received March 14, 1984.* This work was supported by NSF Grant PCM 83-03374.

0006-2960/84/0423-5439$01.50/0