Base-Sequence Dependence of Noncovalent Complex Formation and Reactivity of Benzo[a]pyrene Diol Epoxide with Polynucleotides†

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ABSTRACT: The base-sequence selectivity of the noncovalent binding of (±)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) to a series of synthetic polynucleotides in aqueous solutions (5 mM sodium cacodylate buffer, 20 mM NaCl, pH 7.0, 22 °C) was investigated. The magnitude of a red-shifted absorbance at 353 nm, attributed to intercalative complex formation, was utilized to determine values of the association constant K_{ic}. Intercalation in the alternating pyridine–purine polymers poly(dA-dT)·(dA-dT) (K_{ic} = 20000 M^{-1}), poly(dG-dC)·(dG-dC) (4200 M^{-1}), and poly(dA-dC)·(dG-dT) (9600 M^{-1}) is distinctly favored over intercalation in their nonalternating counterparts poly(dA)·(dT) (780 M^{-1}), poly(dG)·(dC) (1800 M^{-1}), and poly(dA-dG)·(dT-dC) (5400 M^{-1}). Methylation at the 5-position of cytosine gives rise to a significant enhancement of intercalative binding, and K_{ic} is 22000 M^{-1} in poly(dG-m^5dG)·(dG-m^5dC). In a number of these polynucleotides, values of K_{ic} for pyrene qualitatively follow those exhibited by BPDE, suggesting that the pyrenyl residue in BPDE is a primary factor in determining the extent of intercalation. Both BPDE and pyrene exhibit a distinct preference for intercalating within dA-dT and dG-m^5dC sequences. The catalysis of the chemical reactions of BPDE (hydrolysis to tetrols and covalent adduct formation) is enhanced significantly in the presence of each of the polynucleotides studied, particularly in the dG-containing polymers. A model in which catalysis is mediated by physical complex formation accounts well for the experimentally observed enhancement in reaction rates of BPDE in the alternating polynucleotides; however, in the nonalternating polymers a different or more complex catalysis mechanism may be operative. Finally, complex formation and catalysis at different binding sites are related to the level of covalent binding, one of the critical factors in the expression of the mutagenic and tumorigenic potentials of BPDE and related compounds in cells.

Polycyclic aromatic hydrocarbons (PAH) require metabolic activation to express their mutagenic and tumorigenic potentials (Conney, 1982; Singer & Grunberger, 1983). The active metabolites of such PAH compounds are metastable diol epoxide derivatives that are known to bind noncovalently (Harvey & Geacintov, 1988) and covalently (Harvey, 1981; Jeffrey, 1985) to DNA. The mutagenic activities and the initiating step in the complex process of tumorigenesis are believed to involve the covalent binding of the diol epoxide derivatives to cellular DNA (Brookes & Osborne, 1982; Stevens et al., 1985).

Benzo[*a*]pyrene is one of the most widely studied PAH carcinogens; its biologically active form is known to be the metabolite *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene or BPDE[1] (Conney, 1982). The modes of interactions of BPDE with DNA in aqueous solution model systems have been studied extensively (Geacintov et al., 1981, 1982, 1984a; MacLeod & Selkirk, 1982; MacLeod & Zachary, 1985a,b; Michaud et al., 1983; Meehan & Bond, 1984; Shahbaz et al., 1986; Islam et al., 1987). Depending on the stereoisomer of BPDE, only 15% or less of the diol epoxide molecules bind covalently to DNA, while the remainder are hydrolyzed to the tetrols 7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxybenzo[*a*]pyrene (BPT); both of these reactions are catalyzed by nucleic acids.

By use of stopped-flow mixing experiments (Geacintov et al., 1981), it has been shown that noncovalent BPDE–DNA complexes are formed on time scales of less than 5 ms, while the kinetics of the covalent binding reaction take place on time scales of minutes. The primary target site for covalent binding of the highly tumoerigenic (+) enantiomer of BPDE to DNA is the exocyclic amino group of deoxyguanine (Jeffrey, 1985); the less active (−) enantiomer binds also to O6 of dG and to the exocyclic amino group of deoxyadenine (Meehan & Straub, 1979; Osborne et al., 1981; Brookes & Osborne, 1982).

The formation of physical BPDE–DNA complexes is accompanied by a significant hypochromicity and a 10-nm red shift in the absorption spectrum of BPDE (Geacintov et al., 1981; MacLeod & Selkirk, 1982; Meehan et al., 1982; MacLeod et al., 1987; Wolfe et al., 1987). On the basis of this behavior as well as on the linear dichroism spectra of these complexes (Geacintov et al., 1984b; Shahbaz et al., 1986), it is generally believed that BPDE forms noncovalent intercalation complexes with DNA. However, upon covalent binding, the tumorigenic (+)-anti enantiomer of BPDE forms covalent adducts whose properties are consistent with external, or solvent-exposed, adducts, while the less active (−) enantiomer forms covalent adducts that have properties similar to those of the noncovalent intercalation complexes (Geacintov et al., 1984b,c; Jernström et al., 1984; Zinger et al., 1987; Kolubayev et al., 1987).

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[1] Abbreviations: BPDE, (±)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BPT, 7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxybenzo[*a*]pyrene.

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Since the conformations of the noncovalent complexes and covalent BPDE–DNA adducts can be quite different from one another, the possible relationships between noncovalent complex formation and covalent adduct formation have been the subject of speculation and discussion (Chen, 1984; Meehan & Bond, 1984; Geacintov et al., 1986a). It has been suggested that the ability of a PAH diol epoxide or structurally related metabolite to form noncovalent complexes with DNA is correlated with its carcinogenic activity (LeBreton, 1985) and, by implication, its ability to form covalent adducts with DNA. Indeed, a series of mutagenicity and tumorigenicity experiments with more or less bulky alkyl substituents at the 1-position of BPDE appear to be in agreement with this hypothesis (Harvey et al., 1985).

In order to clarify the relationships between physical complex formation, reaction kinetics, and covalent binding, we have studied the base composition and sequence dependence of these factors utilizing BPDE and a series of synthetic polynucleotides of defined base composition and sequence.

Materials and Methods

Racemic BPDE was prepared by methods previously published (Harvey & Fu, 1978). The synthetic polynucleotides were purchased from P-L Biochemicals (Pharmacia, Piscataway, NJ). In the initial experiments these polymers were treated with S_1 nuclease, proteinase K, and phenol extraction, followed by exhaustive dialysis against 5 mM sodium cacodylate buffer containing 50 μM EDTA and dialysis against the buffer solution utilized in all subsequent experiments here (5 mM sodium cacodylate, 20 mM NaCl, pH 7.0). The relative absorbances A_{250}/A_{260} and A_{280}/A_{260} and the CD spectra were the same in the treated and the untreated samples and were in agreement with standard values (Fasman, 1978); furthermore, the ability of BPDE to form noncovalent complexes and covalent adducts with these polynucleotides were also the same in the treated and untreated batches. Consequently, in most of the experiments, the polymers were only dialyzed extensively before use as described. Poly(dG)·(dC) tends to form aggregates and was thus treated before use according to the methods described by Wilson et al. (1986). The concentrations of the polymers, expressed in moles of nucleotides, were determined from the absorbances of the solutions at or near 258 nm by utilizing the known molar extinction coefficients (Wells et al., 1970; Behe & Felsenfeld, 1981).

The methods used for determining the apparent intercalation association constants K_{ic} were described in detail earlier (Shahbaz et al., 1986) and involved measurements of the absorption spectra immediately after BPDE was added to the polymer solutions utilizing a Hewlett-Packard (Model 8451) diode array spectrophotometer. Spectra were recorded at 5–20-s intervals, and the absorbances at the time of mixing at 353 nm (OD_{353}), which are assumed to be proportional to the fraction of intercalated BPDE molecules (Geacintov et al., 1981; Meehan et al., 1982; MacLeod et al., 1982, 1987), were obtained by extrapolation of the decay of OD_{353} as a function of time. This decay, which is due to the conversion of BPDE molecules to tetrols and to covalent adducts, was also utilized to determine values of the pseudo-first-order reaction rate constant k at different polynucleotide concentrations. In order to minimize costs, it was often necessary to work at low polymer concentrations; in such cases it was convenient to utilize a fluorescence kinetic method in order to determine k (Geacintov et al., 1982).

The fraction f_{cov} of initially added BPDE molecules that bind covalently to the polymers was estimated spectrophotometrically. However, direct estimates of f_{cov}, based on the absorption spectra of the covalently bound pyrene residues in the different polynucleotides, were not feasible because the molar extinction coefficients of the bound chromophores are unknown. Therefore, an acid hydrolysis method initially described by Rahn et al. (1982) was modified in order to hydrolyze the adducts to BPT. First, the equilibrated BPDE–polynucleotide reaction mixtures were repeatedly extracted with diethyl ether to remove the BPT molecules; subsequently, the adducts were digested in 0.12 N HCl at 80 °C for 2 h. Control experiments with known amounts of BPT added to polymer solutions showed that the pyrene chromophore was not destroyed during such relatively mild acid hydrolysis treatments, while allowing for a destruction of the secondary structure of the BPDE-modified nucleic acids and release of BPT. The quantity of liberated BPT molecules was then estimated spectrophotometrically, and f_{cov} was calculated.

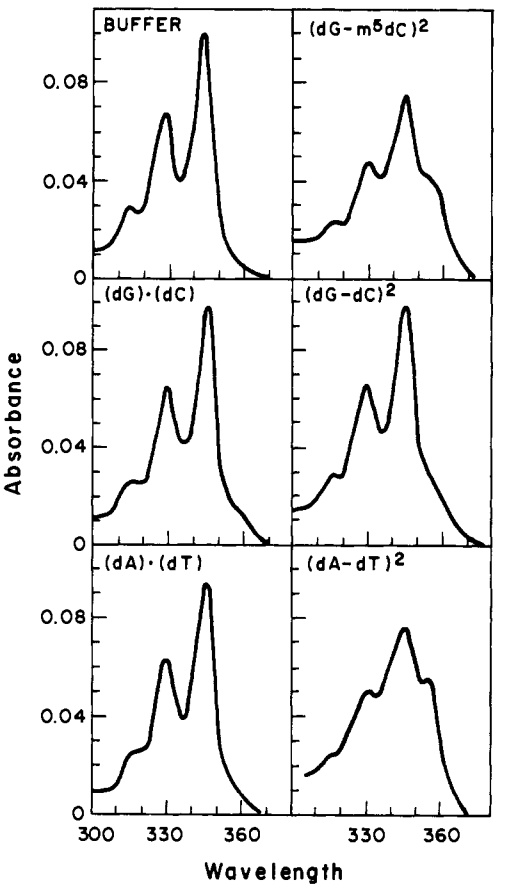

FIGURE 1: Absorption spectra of 3.3 μM aqueous solutions (5 mM sodium cacodylate, 20 mM NaCl, pH 7.0, 22 °C) of BPDE. Concentrations of polynucleotides (nucleotide concentrations): poly(dG)·(dC), 0.11 mM; poly(dA)·(dT), 0.34 mM; poly(dG-m^{5}dC)·(dG-m^{5}dC), 0.10 mM; poly(dG-dC)·(dG-dC), 0.15 mM; poly(dA-dT)·(dA-dT), 0.079 mM.

Results

The absorption spectra of 3.4 μM solutions of BPDE containing approximately comparable concentrations of the different polymers are shown in Figure 1. The formation of noncovalent BPDE–DNA complexes is accompanied by an increase in the absorbance above 350 nm, the extent of which varies from polymer to polymer. The magnitude of this absorbance change ($\Delta[OD]_{353}$) increases with increasing polynucleotide concentration. The apparent intercalative association constant K_{ic} can be estimated by plotting the reciprocal of the absorbance change at 353 nm as a function of 1/[N]

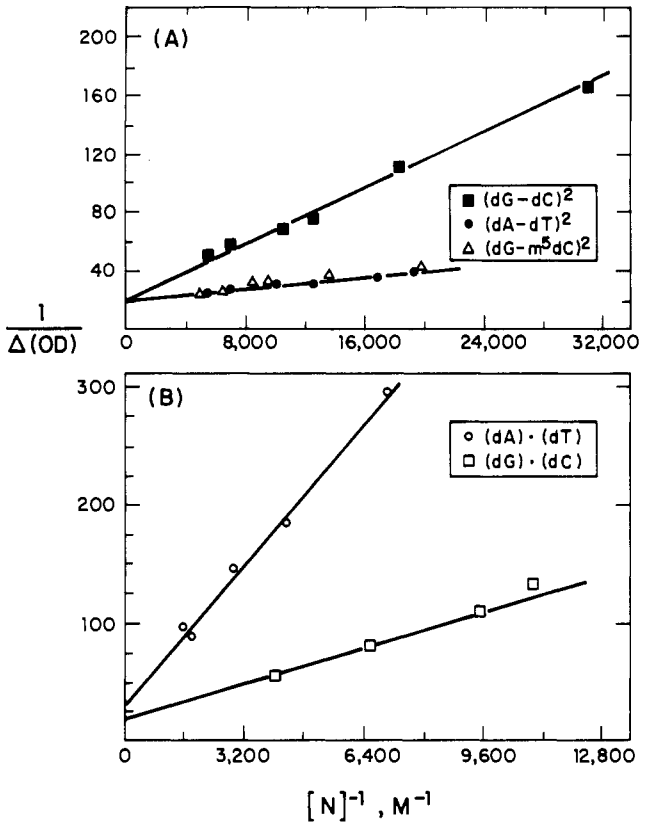

FIGURE 2: Double-reciprocal plot of the absorbance at 353 nm as a function of the polynucleotide concentration according to eq 1. The solid lines represent fits of eq 1 to the data calculated according to a least-squares linear regression program.

(where [N] is the concentration of the polymer expressed in moles of nucleotides) according to the double-reciprocal equation (Benesi-Hildebrand, 1949)

$$\frac{1}{[\Delta OD]_{353}} = \frac{1}{\Delta\epsilon[E]} + \frac{1}{\Delta\epsilon[E]}\frac{1}{K_{ic}}\frac{1}{[N]} \qquad (1)$$

The concentration of diol epoxide molecules is denoted [E], and $\Delta\epsilon$ is the change in the molar extinction coefficient of BPDE upon intercalative complex formation. This equation is valid when nearest-neighbor exclusion effects (McGhee & Von Hippel, 1974) can be ignored; under the conditions of our experiments in which the number of bound molecules/base pair is below 0.02, the use of such simple binding isotherms is justified (MacLeod et al., 1987). Typical plots of the experimental data in terms of eq 1 are shown in Figure 2. The values of K_{ic} were obtained from the slopes and intercepts of these plots and are listed in Table I.

The value of the pseudo-first-order reaction constants k_h in buffer in the absence of nucleic acids is $(1.0 \pm 0.1) \times 10^{-3}$ s^{-1}; however, k increases with increasing polynucleotide concentration in all cases (Figure 3). The catalytic effect is more pronounced in the cases of the alternating purine–pyrimidine copolymers containing dG, and the values of k seem to tend toward saturation at the higher polynucleotide concentrations (Figure 3A); this type of behavior is observed in native DNA as well (Geacintov et al., 1982; MacLeod & Selkirk, 1982; Islam et al., 1987). In the case of the nonalternating polynucleotides, and in the same concentration range, k increases linearly with increasing polymer concentration (Figure 3B). Among these purine–pyrimidine homopolymers, poly(dA-dG)·(dT-dC) exhibits the greatest catalytic effect, followed by poly(dG)·(dC) and poly(dA)·(dT) in that order. Overall, these results are in qualitative agreement with the data of

Table I: Intercalative Noncovalent Association Constant (K_{ic}) for Pyrene and BPDE and Levels of Covalent Binding (f_{cov}) of BPDE in Different Polynucleotides[a]

polynucleotide	pyrene K_{ic}[b] (M^{-1})	BPDE K_{ic}[b] (M^{-1})	BPDE f_{cov}[c]
poly(dG-m⁵dC)·(dG-m⁵dC) (B-form)	22000	22000	0.25
poly(dG-m⁵dC)·(dG-m⁵dC) (Z-form)		<500[d]	0.13[d]
poly(dG-dC)·(dG-dC)	3200	4200	0.18
poly(dA-dC)·(dG-dT)	7400	9600	0.10
poly(dA-dT)·(dA-dT)	41000	20000	<0.02
poly(dA)·(dT)	1700	780	<0.01
poly(dG)·(dC)		1800	0.12
poly(dA-dG)·(dT-dC)		5400	0.13

[a] 5 mM sodium cacodylate buffer, 20 mM NaCl, 22 °C, pH 7.0. All listed values are within ±10%. [b] Determined from the absorbance increases at 353 nm and analysis of data in Figure 2 according to eq 1. [c] Fraction of BPDE molecules that bind covalently to the polynucleotides. [d] Moussaoui et al. (1985).

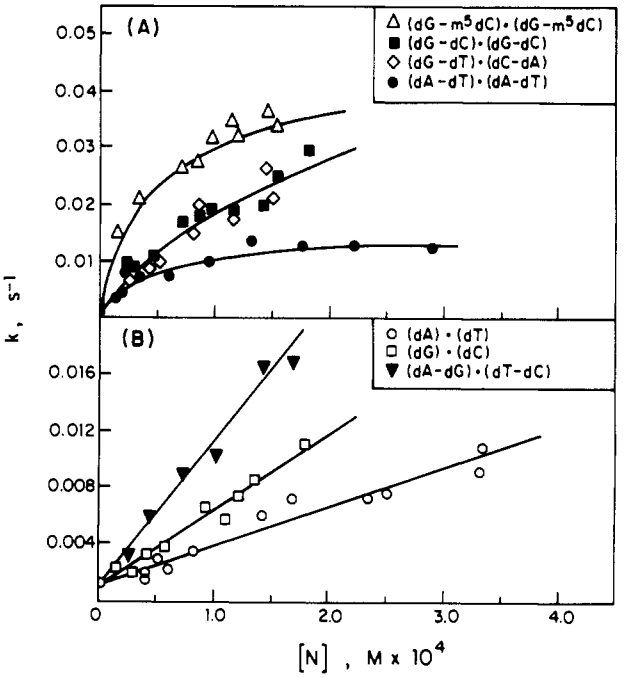

FIGURE 3: Dependence of the overall reaction rate constant k on the polynucleotide concentration. In (A) the solid lines represent visual aids only; in (B) the lines represent linear fits of eq 7 to the data.

MacLeod and Zachary (1985a) and Islam et al. (1987), who showed that dG is more effective than dA in catalyzing the hydrolysis of BPDE to tetrols.

The level of covalent binding of BPDE to different polynucleotides is listed in Table I; as expected, since dG is the primary target base for covalent binding in native DNA (Jeffrey, 1985), f_{cov} is highest in the dG-containing polymers and lowest in the alternating and nonalternating dA-dT polymers.

DISCUSSION

Base-Sequence Preferences of Noncovalent Complex Formation. The base-sequence preferences of intercalative complex formation of electrically neutral polycyclic aromatic hydrocarbons, in contrast to that of cationic drug molecules, has received relatively little attention.

Many cationic drug intercalators exhibit slight to pronounced preferences for binding at dG-dC sites (Wilson et al., 1985). Molecules of this type include ethidium (Krugh et al., 1975; Reinhardt & Krugh, 1978; Baguley & Falkenhaug, 1978; Doglia et al., 1983), quinacrine (Baldini et al., 1981),

acridine derivatives (Müller & Crothers, 1975; Young & Kallenbach, 1981; Atwell et al., 1984), actinomycin (Müller & Crothers, 1968), and other antibiotic/antitumor drugs (Jones et al., 1987). Of special significance are the observations of Krugh and his co-workers that ethidium displays a significant preference for binding at pyridine(3′–5′)purine rather than at purine(3′–5′)pyrimidine sequences (Krugh et al., 1975; Reinhardt & Krugh, 1978). This preference has been attributed to the energy requirements of local unstacking and unwinding in the process of creating an intercalation site (Ornstein & Rein, 1979; Broyde & Hingerty, 1979; Miller et al., 1980).

A smaller number of drug intercalators appear to prefer alternating dA-dT sequences over alternating dG-dC sequences; these include, for example, daunomycin (Chaires, 1983), tilorone (Strum, 1982), and propidium (Wilson et al., 1986). Furthermore, Wilson et al. (1985, 1986) have recently synthesized naphtothiophene, anthracene, and phenanthrene derivatives that contain amide and ester side chains with cationic groups, and these exhibit significant preferences for binding noncovalently to dA-dT sites. Chen (1983, 1984) has shown qualitatively, utilizing the red shift in the absorption spectrum of BPT induced by intercalative complex formation (Ibanez et al., 1980), that these tetrol molecules (as well as pyrene) intercalate significantly in poly(dA-dT)·(dA-dT), only slightly in guanine-containing purine–pyrimidine alternating copolymers, and negligibly in the homopolymers poly(dA)·(dT) and poly(dG)·(dC). Similar conclusions were reached in the case of one other metabolite model compound of benzo[*a*]-pyrene, 7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (Yang et al., 1983). It is evident from the relative values of K_{ic} (Table I) that BPDE exhibits similar base-sequence preferences. Thus, in contrast to many cationic drug molecules, polycyclic aromatic derivatives that lack ionic sites or groups exhibit a distinct preference for intercalating at alternating dA-dT rather than at dG-dC sequences.

Qualitatively, the values of K_{ic} for BPDE and for pyrene follow the same general trends in the different polynucleotides (Table I), being highest in poly(dG-m^{5}dC)·(dG-m^{5}dC) and poly(dA-dT)·(dA-dT) and lowest in poly(dA)·(dT). The preference of BPDE for intercalating at dA-dT or dG-m^{5}dC sequences seems to be a property of the aromatic pyrenyl chromophore rather than the oxide and hydroxyl group substituents of BPDE; however, the steric properties of these substituents in different stereoisomers of polycyclic aromatic diol epoxide molecules can also modulate the magnitude of K_{ic} by factors of 2 (Shahbaz et al., 1986) or more (Carberry et al., 1988).

The association constant K_{ic} is 5 times greater in poly(dG-m^{5}dC)·(dG-m^{5}dC) than in poly(dG-dC)·(dG-dC); this effect is not due to variations in the conformations of these two polynucleotides since both are in the B-form under the relatively low ionic strength conditions of our experiments (Rich et al., 1984). Differences in the polarizabilities of the nucleic acid bases, as suggested by Müller and Crothers (1975), also do not seem to influence the magnitudes of K_{ic} in these two polynucleotides since they differ chemically from one another only by the presence of a methyl group. This methyl group interacts with water molecules in the major groove, thus destabilizing the B-form of poly(dG-m^{5}dC)·(dG-m^{5}dC) (Wang et al., 1985); such a partial destabilization may also facilitate the creation of an intercalation site, thus enhancing the value of K_{ic} in the methylated polymer. Furthermore, with the methyl group at the 5-position of dC, poly(dG-m^{5}dC)·(dG-m^{5}dC) somewhat resembles poly(dA-dT)·(dA-dT). These two characteristics suggest that hydrophobic effects may be important in the physical intercalation of BPDE with the pyrenyl ring protruding into the major groove. Theoretical considerations indicate that the pyrene ring systems of intercalated BPDE molecules can indeed protrude into both the minor and the major groove (Miller et al., 1981; Subbiah et al., 1983).

In general, the values of K_{ic} are consistently higher in the alternating than in the nonalternating purine–pyrimidine sequence polymers (Table I). These differences may be understood in terms of the energy requirements for creating intercalation sites in B-DNA, which involves both unstacking of base pairs to accommodate the intercalants and unwinding of the double helix. The energy required for creating such intercalation sites is lower for alternating pyrimidine(3′–5′)-purine than for nonalternating homocopolymer and purine-(3′–5′)pyrimidine sequences (Ornstein & Rein, 1979; Miller et al., 1980; Lybrand & Kollman, 1985). The exact reasons for the experimentally observed preferences of the intercalation of pyrene and BPDE between alternating dA-dT rather than between alternating dG-dC sequences remain to be elucidated. Theoretical calculations performed so far [for example, Miller et al. (1980)] cannot adequately account for this base selectivity.

The association constant K_{ic} of BPDE is significantly smaller in poly(dA)·(dT) than in the other polynucleotides. In the case of pyrene, it is nearly 25 times smaller in this polymer than in the alternating poly(dA-dT)·(dA-dT) copolymer. Unusually small values of the physical binding constants in poly(dA)·(dT) have also been observed in the cases of ethidium (Bresloff & Crothers, 1981), daunomycin (Chaires, 1983), and propidium (Wilson et al., 1985). These low binding affinities in poly-(dA)·(dT) appear to be related to the unusual structural characteristics of this polymer in solution (Wilson et al., 1985).

The conformations of the polynucleotides in solution can also play an important role in determining the value of K_{ic} in the dG-dC polymers as well. For example, it was previously noted that BPDE does not form any spectroscopically discernible physical complexes with the Z-form of poly(dG-m^{5}dC)·(dG-m^{5}dC) (Moussaoui et al., 1985), although the affinity for the B-form is quite pronounced (Table I). However, such conformational effects cannot account for the differences in K_{ic} observed in the case of the homo- and hetero-dG-dC polymers, since both exist in the B-form in solution under conditions of low ionic strength (Shakked & Rabinovich, 1986).

Relationships between Physical Complex Formation and Reaction Rate Constants. In aqueous solutions of native double-stranded calf thymus DNA, both the reaction rate constant k and the fraction of intercalated BPDE molecules increase with increasing DNA concentration in a parallel manner (Geacintov et al., 1982); similar results were obtained with other PAH diol epoxides (Kim et al., 1984; Carberry et al., 1988). It was suggested that catalysis of the chemical reactions of these diol epoxide molecules occurs at noncovalent DNA binding sites. According to this model, the dependence of k on the polymer concentration [N] is given by (Geacintov et al., 1982; Michaud et al., 1983)

$$k = k_h X_f + k_3 X_b \tag{2}$$

where k_h and k_3 are the reaction rate constants of free and bound molecules, respectively. X_b and X_f are the fractions of physically bound and free BPDE molecules, respectively, defined as

$$X_b = K[N]/(1 + K[N]) \tag{3a}$$

$$X_f = 1/(1 + K[N]) \tag{3b}$$

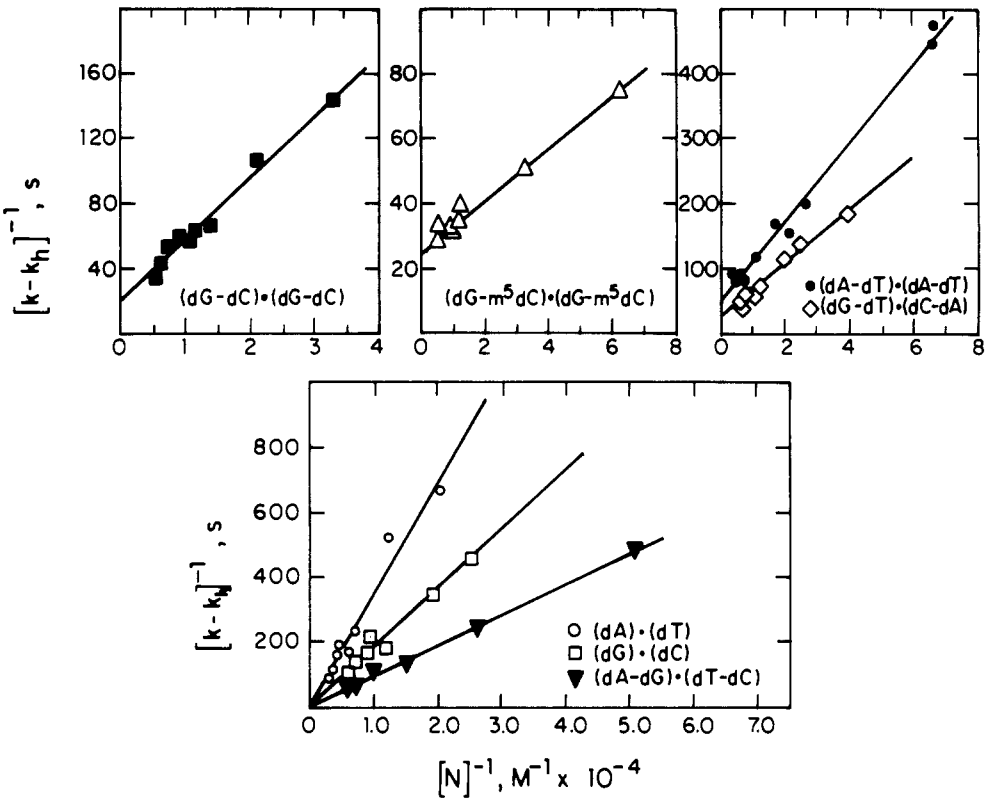

FIGURE 4: Double-reciprocal plot of the reaction rate constant k as a function of the polynucleotide concentration. The solid lines represent fits to the data points calculated according to eq 4.

where $K = k_1/k_2$ is the overall noncovalent association constant, while k_1 and k_2 are the effective rate constants of formation and dissociation, respectively, of the noncovalent complexes.

The values of K are best determined by inverting eq 2 and replotting the experimental data shown in Figure 3 according to the double-reciprocal relation (Kim et al., 1986)

$$\frac{1}{k - k_h} = \frac{1}{k_3 - k_h} + \frac{1}{(k_3 - k_h)K}\frac{1}{[N]} \qquad (4)$$

Typical plots of the data of Figure 3 for the alternating and nonalternating purine–pyrimidine copolymers are shown in Figure 4, parts A and B, respectively. Generally, k_3 can be determined from the values of the intercepts and the known value of k_h, while K can be deduced from the (intercept)/(slope) ratios.

(A) Alternating Purine–Pyrimidine Polymers. The values of k appear to level off with increasing nucleic acid concentration, in agreement with the predictions of eq 2 and the results obtained with native DNA (Geacintov et al., 1982). The intercepts $(k_3 - k_h)^{-1}$ of the double-reciprocal plots in Figure 4A lie in the range of 20–30, and the corresponding values of k_3 are listed in Table II. The presence of dG is correlated with higher values of k_3, as expected form the results of MacLeod and Zachary (1985a). However, even in the case of poly(dA-dT)•(dA-dT), k_3 is at least 10 times greater than k_h.

(1) Comparisons of the Apparent Association Constants K and K_{ic}. The values of K deduced from Figure 4 (top three panels) are listed in Table II and are compared to the corresponding values of the intercalative association constants K_{ic}. Considering the magnitudes of the combined experimental errors involved, the values of K and K_{ic} are generally close to one another; possible exceptions are poly(dG-m^{5}dC)•(dG-m^{5}dC) and poly(dA-dT)•(dA-dT) for which the K/K_{ic} ratios are 1.5 ± 0.25 and 0.4 ± 0.25, respectively. In native DNA

Table II: Apparent Association Constant (K) and Reaction Rate Constant k_3 for BPDE in Different Polynucleotides Determined According to Eq 3 and 4, Respectively[a]

polynucleotide	K[b] (M^{-1})	K/K_{ic}	k_3[c] (s^{-1})	slope[d] (M^{-1} s^{-1})
poly(dG-m^{5}dC)•(dG-m^{5}dC) (B-form)	33000	1.5 ± 0.25	0.037	
poly(dG-dC)•(dG-dC)	5300	1.3 ± 0.25	0.052	
poly(dA-dC)•(dG-dT)	6800	0.7 ± 0.25	0.037	
poly(dA-dT)•(dA-dT)	8200	0.4 ± 0.25	0.017 ± 0.004	
poly(dA)•(dT)	<800	<1.0	>0.035	28
poly(dG)•(dC)	<1700	<1.0	>0.032	55
poly(dA-dG)•(dT-dC)	<1700	<0.3	>0.059	100

[a] 5 mM sodium cacodylate buffer, 20 mM NaCl, 22 °C, pH 7.0. All listed values are within ±15% unless noted otherwise. [b] Determined from the dependence of the reaction rate constant k on the polynucleotide concentration (Figure 4 and eq 4). [c] Reaction rate constant at infinite polynucleotide concentration (eq 6). [d] From Figure 3B.

$K \approx K_{ic}$, as predicted by the simple kinetic model on which eq 2 is based (Geacintov et al., 1982). However, there are other factors that can give rise to differences in the magnitudes of K and K_{ic}.

It should be recalled that K_{ic} is a measure of only those binding sites that are characterized by red shifts in the absorption spectra of BPDE, while K is a measure of all binding sites that are active in the catalysis of BPDE. For example, the existence of external noncovalent DNA binding sites for BPDE has been previously postulated (Chen, 1984); if these putative external binding sites are characterized by small or nonexistent absorption red shifts and if they are catalytic in nature, then K is predicted to be larger in magnitude than K_{ic}, as appears to be the case in poly(dG-m^{5}dC)•(dG-m^{5}dC).

However, independent evidence for the existence of such sites is lacking at this time.

Values of K smaller than K_{ic} have been previously observed in only one other case (BPDE in Tris buffer–DNA solution, pH 9.5; Shahbaz et al., 1986). This effect can be accounted for if buffer molecules, which catalyze the reactions of DNA-bound BPDE molecules (Meehan & Bond, 1984), also bind to the DNA molecules. The overall rate constant k is then proportional to both the fraction of bound BPDE and bound buffer molecules (Geacintov, 1986a); if the equilibrium binding constant for buffer molecules is much lower than K for the BPDE molecules, k will increase at a slower rate with increasing DNA concentration than predicted by eq 2 alone (Shahbaz et al., 1986).

(*2*) *Interpretation of the Catalysis Rate Constant* k_3. The ratio k_3/k_h is an empirical measure of the catalytic effect of the nucleic acids on the reactivity of BPDE. When there are multiple binding sites of type **i** (intercalative as well as external) and catalysis of BPDE can occur at each of these sites with a rate constant $k_3(\mathbf{i})$, k_3 is defined as (Geacintov, 1986a)

$$k_3 = \sum_{\mathbf{i}} k_3(\mathbf{i})(K(\mathbf{i})/K) \tag{5}$$

where

$$K = \sum_{\mathbf{i}} K_{\mathbf{i}} \tag{6}$$

if the binding sites are considered to be independent of one another (Klotz, 1974). In this model, k_3 is equal to a sum of site-specific reaction rate constant $k_3(\mathbf{i})$, each weighted by the probability $K(\mathbf{i})/K$ that the BPDE molecules reside at each particular site **i**. It is evident that the values of k_3 derived experimentally and presented in Table II cannot be interpreted in a unique manner because of the heterogeneity of catalytic, noncovalent binding sites.

(*B*) *Nonalternant Polynucleotides*. In the cases of the nonalternating polymers, the straight lines obtained by fitting the data to a least-squares linear regression program pass through zero (Figure 4B); the exact values of these intercepts are 0.24 for poly(dA)·(dT), −0.04 for poly(dG)·(dC), and 0.30 in the case of poly(dA-dG)·(dT-dC). If it is assumed that the enhanced reaction kinetics of BPDE in solutions of these nonalternant polynucleotides is governed by the mechanism of eq 2, then the linearity of the k vs [N] plots (Figure 3B) suggests that $K[\mathrm{N}] < 0.25$ in the polymer concentration ranges studied; the basis for this estimate is that deviations from linearity of 20% at the highest experimental value of [N] in Figure 3B would have been recognizable. Utilizing these maximum values of [N], upper values of K within the range 800–1700 are estimated for the three nonalternating polynucleotides (Table II). Within this polymer concentration range

$$k \cong k_h + k_3 K[\mathrm{N}] \tag{7}$$

Lower limits of the values of k_3 can be estimated from the slopes in Figure 3B and the upper limits of K and are also listed in Table II.

The fact that the straight lines in Figure 4B pass through zero suggests that $k_3 \gg k_h$ and that the k_3 values are probably significantly higher than the lower bound estimates provided in Table II. Therefore, it cannot be excluded that the mechanisms of catalysis of BPDE are different in the nonalternating than in the alternating polynucleotides. One possibility is that in the nonalternating polymers diffusional collisions between free BPDE and the polymers (rate constant k_D) play a significant role in catalysis. In that case, the term $k_D[\mathrm{N}]X_f$ would be added to the right side of eq 2, and the effective value of k_3 would become $(k_D/K + k_3')$; thus, two different mechanisms of catalysis could be operative simultaneously, one involving free BPDE molecules and a collisional encounter with the nucleic acids, the other involving catalysis of BPDE molecules at noncovalent binding sites (rate constant k_3'). The experimentally observed values of k_3 could then be quite large, since the values of k_D can be as high as 10^9–10^{10} M^{-1} s^{-1}.

It is interesting to note that a similar linear concentration dependence of the reaction rate constant and a high apparent rate constant of catalysis were also noted by Islam et al. (1987) for BPDE in solutions of the homopolymer poly(G).

Base-Sequence Preferences of Noncovalent Complex Formation and Covalent Adduct Formation. The fractions (f_{cov}) of BPDE molecules that bind covalently to the different polynucleotides are listed in Table I. Since covalent binding of BPDE occurs preferentially at dG (Jeffrey, 1985), while noncovalent intercalative complex formation occurs preferentially at dA-dT sites, there is no direct apparent relationship between noncovalent intercalation and the level of covalent product formation.

The relationship between f_{cov} and noncovalent complex formation is, within the context of the model involving the formation of prereaction physical complexes (Geacintov, 1986a)

$$f_{cov} = \frac{(k_h)^{-1}[\sum y_{\mathbf{i}} k_3(\mathbf{i})K(\mathbf{i})]}{1 + (k_h)^{-1}[\sum_{\mathbf{i}} k_3(\mathbf{i})K(\mathbf{i})][\mathrm{N}]} \tag{8}$$

where the summations are taken over all types of binding sites **i**, and $y_{\mathbf{i}}$ are the fractions of BPDE molecules that undergo the covalent binding reaction (rather than undergoing hydrolysis to tetrols) at a given physical binding site **i**. At these sites, the level of covalent binding depends not only on $y_{\mathbf{i}}$ but also on the product $k_3(\mathbf{i})K_{\mathbf{i}}$. Thus, while the overall level of covalent binding depends on the site-specific noncovalent association constants $K(\mathbf{i})$, this dependence may be weak because these terms occur in both the numerator and denominator of eq 8 and because the $y_{\mathbf{i}}k_3(\mathbf{i})$ terms may be the dominant factors. Since both catalysis of reactions of BPDE (MacLeod & Zachary, 1985a) and covalent binding are favored at dG, the lower values of K_{ic} at dG-dC sites are compensated by higher values of y and k_3. At dA-dT sites, exactly the opposite situation prevails, with high probability of formation of noncovalent intercalation complexes but with lower probabilities of chemical reaction.

Implications for Reactions of BPDE with Native DNA. In the case of native DNA with 10 distinct intercalation sites and possibly additional nonintercalative and noncovalent binding sites, a simple picture of the covalent binding of BPDE to DNA can be proposed. Noncovalent complex formation is preferred at dA-dT sites [but not in segments containing runs of nonalternating (dA)·(dT) sequences], and thus BPDE molecules reside a greater fraction of the time at these sites where chemical reactions with dA-dT base pairs are not favored. However, the residence time at each physical binding site is limited (<7 ms, S. K. Kim and N. E. Geacintov, unpublished observations); thus, diffusion of BPDE from site to site can occur before the slower chemical reactions occur. The probability of forming a chemical adduct is highest at sites containing dG, and a given BPDE can sample many different binding sites during its lifetime of minutes in an aqueous environment containing DNA. Under these conditions, the dependence of f_{cov} on the physical binding constants can be very low or even nonexistent (Geacintov, 1986a). An example of this effect is the relatively high f_{cov} value in the case of the

Z-form of poly(dG-m^{5}dC)·(dG-m^{5}dC), even though physical binding is drastically reduced as this polymer is transformed from the B-form to the Z-form under conditions of near-identical ionic strength (Table I; Moussaoui et al., 1985).

Finally, from eq 8, ratios of covalent adducts formed at different sites in heterogeneous DNA can be estimated; the ratios of products formed at two particular sites a and b are

$$\frac{y_a}{y_b}\frac{k_3(a)}{k_3(b)}\frac{K_a}{K_b} \quad (9)$$

Such an expression might be a useful starting point for analyzing DNA adduct distributions if the factors on the right-hand side of this equation can be evaluated independently by using oligonucleotides of well-defined base composition and sequence.

Intercalation, Binding to Cellular DNA, and Biological Activity. The covalent binding of BPDE and similar molecules to DNA is one of the critical factors in the expression of the mutagenic and tumorigenic potentials of these molecules. In aqueous model systems, the dependence of the level of covalent binding of BPDE to DNA on the noncovalent intercalation constant K_{ic} is generally predicted to be weak (eq 8). However, in situ, a much stronger dependence might be observable. In fact, Harvey et al. (1985) have found that the mutagenic and tumorigenic activities of BPDE are significantly lowered by bulky alkyl substituents that inhibit the formation of noncovalent intercalation complexes (Paulius et al., 1985). In a cellular environment, there are many competing reaction pathways that limit the binding of BPDE to DNA; for example, the diol epoxide molecules react with water, glutathione, and other cellular nucleophiles and form noncovalent complexes not only with DNA but with other cellular macromolecules as well. The relative distributions of polycyclic aromatic diol epoxide molecules in the aqueous phases of the cell and at hydrophobic binding sites in macromolecules other than DNA should depend on the relative association constants. The higher the value of K_{ic} (or K), the higher the probability that the diol epoxide molecules will be noncovalently bound with DNA, and thus the greater the probability that these molecules will covalently bind to DNA. The tumorigenic and mutagenic potentials of BPDE and related molecules might thus in fact depend on the relative magnitudes of K_{ic}, which in turn determine the probabilities of formation of noncovalent, prereaction diol epoxide–DNA complexes (Geacintov, 1986b).

Registry No. BPDE, 63323-31-9; poly(dG-m^{5}dC), 51853-63-5; poly(dG-dC), 36786-90-0; poly(dA-dC)·poly(dG-dT), 55684-99-6; poly(dA-dT), 26966-61-0; poly(dA)·(dT), 24939-09-1; poly(dG)·(dC), 25512-84-9; poly(dA-dG)·(dT-dC), 53232-17-0.

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