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BASE SEQUENCE EFFECTS ON INTERACTIONS OF AROMATIC MUTAGENS WITH DNA

Progress Report

for the period March 1, 1991 - August 31, 1991

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MASTER

INTRODUCTION AND OVERVIEW

This report is a summary of research performed during the period March 1, 1991 to August 31, 1991 and supported by DOE Grant No. DE-FG02-88ER60674. This report covers the first six-months period of the first year in a three-year project period, which expires on February 28, 1994.

OBJECTIVES

The importance of base sequence on the local DNA structure and on gene expression is well known. The chemical binding of mutagenic and tumorigenic metabolites of polynuclear aromatic hydrocarbons (PAH) derivatives to nucleic acids is also base-sequence dependent. The objectives of this research are to systematically determine how the secondary structure of DNA, and the sequences of bases around the modified DNA base, affects the reactivities of selected reactive PAH metabolites and model compounds and the properties of the carcinogen-DNA lesions. The techniques used are based on the synthesis of site-specific and stereochemically defined adducts derived from monomeric, dimeric, trimeric, and higher deoxyoligonucleotides of defined base composition and sequence, in order to assess the effects of neighboring bases on reactivities and adduct properties in the single- and double-stranded forms. A variety of spectroscopic and thermodynamic methods are utilized to characterize mono-nucleotide, dinucleotide, trinucleotide, etc. adducts to characterize the interactions of the mutagenic bulky lesions with neighboring DNA bases. Molecular modeling techniques, employing a range of available software, are being utilized to correlate the experimental results with energy-minimized molecular views of the PAH metabolites bound to different sequences in nucleic acids.

SUMMARY OF PROGRESS WITHIN THE FIRST SIX-MONTH PERIOD

I. Within this period, we have completed our investigations on the thermodynamic characteristics and base sequence dependence of duplex formation of benzo[a]pyrene diol epoxide (BPDE) DNA adducts. Different 11-mer oligonucleotides containing covalently bound BPDE moieties at the exocyclic amino group of a single guanine base were utilized in these studies. Last year, in the three-year progress report, some preliminary data were discussed. A final account is provided here.

II. New techniques were developed for assessing the preferred orientations of the enantiomers of (+)-BPDE and (-)-BPDE relative to the 5'---->3' polarity of DNA strands; these investigations were prompted by predictions derived from our computer modeling studies.

III. Significant progress was made towards synthesizing BPDE-adenine adductsin base sequence-specific oligonucleotides.

IV. We failed, on the other hand, to synthesize nitrosopyrene-oligonucleotide adducts because of intrinsic low reactivities of the nitrenium derivative ions with oligonucleotides. Nature was against us in this effort. Therefore, this particular goal had to be abandoned.

In this Progress Report, we focus on subprojects I and II in some detail.

I. THERMODYNAMICS OF HELIX-COIL TRANSITIONS IN STEREOSPECIFIC BENZO[a]PYRENE DIOL EPOXIDE-OLIGONUCLEOTIDE ADDUCTS.

INTRODUCTION

The ultimate mutagenic and carcinogenic metabolites of benzo[a]pyrene are the bay region diol epoxides [1]. There are two diastereomers, and each of these is resolved into a pair of enantiomers. The two (+) and (-) (or R,S,S,R and S,R,R,S) enantiomers of the anti diastereomer trans-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) are of particular interest for structure-biological activity correlation studies because (+)-BPDE is highly tumorigenic while the (-) enantiomer is not (reviewed in [1]). We have recently succeeded in synthesizing stereospecific BPDE-oligonucleotide adducts by direct synthesis methods [2,3]. Both (+)- and (-)-BPDE give rise to N²-guanine-C10 BPDE adducts via trans and cis addition of dG relative to the 9,10-oxirane moiety of BPDE [2,4]. In this section, the temperature dependence of the helix-coil equilibrium is described for (+)-trans, (+)-cis, (-)-trans and (-)-cis adducts derived from the binding of (+)-BPDE and (-)-BPDE to the single dG of the 11-mer oligonucleotide d(CACATGTACAC) (N11) in equimolar solutions of the complementary, or near-complementary oligonucleotide d(GTGTA-X-ATGTG), where X = C, T, A or G.

MATERIALS AND METHODS

The (+)- and (-)-BPDE enantiomers were synthesized according to published methods [5]. The oligonucleotides were synthesized using the phosphoramidite method, purified, reacted with BPDE, and the BPDE-oligonucleotide adducts were purified as described earlier [2,3]. The (-)-cis adducts are obtained in low yields, and also tend to be unstable [2]. Thus, all data given for this adduct are probably less accurate than for the other three. Before measuring the melting curves, equimolar amounts of the modified or unmodified single-stranded oligonucleotides and the complementary (or near-complementary) strand were annealed by heating the samples to 70 °C for about 10 minutes, and then allowing the samples to cool slowly back to room temperature overnight. Absorbances were measured at 260 nm while the temperature was increased at a rate of 0.42 °C/min. The melting point T_m is defined as the temperature at which α , the fraction of strands in the duplex state, is equal to 0.5 [6]. However, T_m is often difficult to determine because of poorly defined baselines. Therefore, T_m was determined from the maxima in plots of the first order derivative $d\alpha/dT$ versus the temperature T . The values of T_m determined by these two methods are generally not identical, but are typically within 1-2 degrees of each other [7]. Experimental T_m values obtained by the derivative method were reproducible to within ± 0.5 °C, as determined from heating, cooling, and reheating cycles. The concentrations of the unmodified oligonucleotide strands were determined by the methods described by Warshaw et al. [8]. The concentration of the single-stranded BPDE-N11 adducts was determined as described by Cosman et al. [2]. All samples were dissolved in 20 mM Na₂HPO₄, 0.1 M NaCl, pH 7.0 buffer solution.

For non-self complementary oligonucleotides in equilibrium:

an equilibrium constant K can be defined which is related to the thermodynamic parameters of duplex formation via the usual equations

$$\Delta G^{\circ} = -RT \ln K \quad (2)$$

and

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ} \quad (3)$$

The thermodynamic parameters can be derived from the T_m values at a number of different strand concentrations (C_T) from plots of

$$1/T_m = (R/\Delta H^{\circ}) \ln C_T + (\Delta S^{\circ} - 0.83R)/\Delta H^{\circ} \quad (4)$$

as described in detail elsewhere [9]. A second method for determining the transition enthalpy (denoted by ΔH_{VH} in this case) involves an analysis of the melting curves according to the equation [9]:

$$\Delta H_{VH} = 6RT_m^2 (d\alpha/dT)_{T=T_m} \quad (5)$$

The van't Hoff enthalpies ΔH° and ΔH_{VH} defined in Eqs. 4 and 5, respectively, should be identical to one another if the single strand-duplex equilibrium defined in Eq. 1 is correct and if this process is cooperative. Thus, by measuring these two enthalpies separately, it is possible to determine whether there are any intermediate base-pairing states involved in the melting process, or whether the melting is a "none or all" transition [6,7].

RESULTS

The melting profiles of the unmodified duplex d(CACATGTACAC)·d(GTGTACATGTG) and all four BPDE-N11 adducts annealed with the natural complement d(GTGTACATGTG) are shown in Fig. 1. The T_m of the unmodified duplex is 43 °C (C_T = 10 μ M). The presence of the bulky BPDE group bound to the exocyclic amino group of guanine leads to a lowering of the T_m in all cases (32 °C, (+)-trans, C_T = 10 μ M; T_m = 34 °C, (+)-cis, 10 μ M; 28 °C, (-)-trans, 8 μ M; 21 °C, (-)-cis, 3 μ M).

The BPDE-N11 modified strands also form duplexes with strands d(GTGTAATGTG) which are complementary to N11 except for the bases opposite the modified dG; the melting curves for the (+)-trans-BPDE-N11 mismatched duplexes are shown in Fig. 2. At a 10 μ M concentration of duplexes, T_m is lower in all cases (26 °C, X = A; 21 °C, X = T; 24 °C, X = G).

The melting points of modified and unmodified duplexes are dependent on the total strand concentration, C_T . Typical plots of T_m vs. $\ln C_T$ are shown in Fig. 4. Thermodynamic parameters ΔG° , ΔH° , and ΔS° , derived from these plots are summarized in Table 1. The enthalpy change ΔH° can also be obtained by converting the melting curves into α vs. T plots, and then evaluating the slope $\partial\alpha/\partial T$ vs. T curves at $T = T_m$ as described above. The enthalpy changes determined by this method are designated as ΔH_{VH} and are compared to the ΔH° values obtained from the slopes of the lines in Fig. 4, in Table 2. The values of T_m and ΔH_{VH} determined for the (+)-trans-BPDE-N11-mismatched oligonucleotide duplexes are summarized in Table 3.

Fig. 1: Melting curves of unmodified (---) and BPDE-modified $d(CACATG^{BPDE}TACAC) \cdot d(GTGTACATGTG)$ (-----) duplexes. A: (+)-trans-BPDE-N²-dG, B: (+)-cis, C: (-)-trans, and D: (-)-cis.

Fig. 2: Melting curves (+)-trans-N²-dG d(CACATGBPDETACAC)·d(GTGTA-XATGTG) duplexes with X = A, T, G, or C.

Fig. 3: Concentration dependence (molarity in terms of total strand concentration) of T_m of $d(\text{CACATG}^{\text{BPDE}}\text{TACAC}) \cdot d(\text{GTGTACATGTG})$ duplexes.

Fig. 4: Absorption spectra of single-stranded (+)-trans-BPDE d(CACAT-G^{BPDE}TACAC) adduct (-----), and its duplexes (————) with d(GTGTA_XATGTG), where X = A, T, G, or C.

	ΔH°	$\Delta\Delta H^\circ$	ΔS°	$\Delta\Delta S^\circ$	ΔG°	$\Delta\Delta G^\circ$
<i>Unmodified</i>	-86	—	-0.25	—	-12	—
<i>(+) TRANS</i>	-91	- 5	-0.27	-0.026	- 9.4	+3.0
<i>(+) CIS</i>	-96	-10	-0.28	-0.037	-11	+1.7
<i>(-) TRANS</i>	-71	+15	-0.21	+0.037	- 8.1	+4.3
<i>(-) CIS</i>	-45	+41	-0.13	+0.12	- 7.0	+5.4

1. Enthalpy and free energy changes are in units of kcal/mol.
2. Entropy change is in units of kcal/mol·K
3. Values are within $\pm 10\%$
4. $\Delta\Delta$ function is defined as the difference between the thermodynamic parameters of the adducts and that of the unmodified duplex.

Table 1. Thermodynamic parameters evaluated from concentration-dependent data (Fig. 4), according to Eq. 3 for unmodified and modified d(CACATG^{BPDZ}TACAC)·d(GTGTACATGTG) duplex formation.

	ΔH° , kcal/mol			
METHOD	$\frac{1}{T}$ vs $\ln C$	SLOPE	PERCENT DIFFERENCE	$\Delta H_{vh}/\Delta H^\circ$
Unmodified	-86 ± 4	-93 ± 5	8	1.10
(+) <i>trans</i>	-91 ± 5	-61 ± 4	33	0.67
(+) <i>cis</i>	-96 ± 5	-69 ± 4	28	0.72
(-) <i>trans</i>	-71 ± 5	-44 ± 4	38	0.62
(-) <i>cis</i>	-45 ± 4	*	*	*

*Insufficient lower baseline in the melting curves prevented accurate determination of α and T_m values.

Table 2. Transition enthalpies evaluated from data treatment according to Eqs. 3 and 4 for unmodified and modified d(CACATG^{BPD}-TACAC)·d(GTGTACATGTG) duplex formation.

(+)trans	T_m °C	ΔH_{vh} kcal/mol
A opposite	26.0 ± 0.5	-65 ± 5
T opposite	20.5 ± 0.5	-92 ± 8
G opposite	23.5 ± 0.5	-86 ± 7
C opposite	32.5 ± 0.5	-61 ± 3

Table 3. Transition enthalpies for (+)-trans-N²-dG d(CACATG^{BPD}-TACAC)·d(GTGTAXATGTG) duplexes with X = A, T, G, or C.

DISCUSSION

The presence of the covalently bound BPDE moieties causes significant destabilizations of the duplexes (Table 1). The T_m are lowered, and the melting process occurs over wider temperature ranges (Fig. 1). In all cases, including the unmodified duplex, the van't Hoff transition enthalpy is negative, and the entropy changes are unfavorable for duplex formation. The destabilization is largest for the (-)-cis duplex ($\Delta\Delta G^\circ = +5.4$ kcal/mol, and appears smallest for the (+)-cis complex. The errors inherent in these types of measurements have been discussed by Telser et al. [10]. The uncertainties of $\pm 10\%$ in the values refer mostly to the absolute values of the thermodynamic parameters listed which stem in part from uncertainties in the absolute concentrations of the strands, and uncertainties in locating the maxima in the derivative $\partial\alpha/\partial T$ curves. Since all measurements were performed identically, however, relative errors in the determinations of ΔG° and ΔH° parameters should be smaller [10]. The transition enthalpy is significantly smaller in the two adducts derived from (-)-BPDE; however, these larger $\Delta\Delta H^\circ$ values are offset by positive $\Delta\Delta S^\circ$ values which combine to produce smaller changes in the free energy of duplex formation (Table 1). In the case of the adducts derived from (+)-BPDE, the changes in the transition enthalpies appear to be small, and the changes in duplex stability appear to be due to less favorable entropy factors in the modified (+)-trans and (+)-cis BPDE-N11 adducts. Recent NMR measurements with a (+)-trans-N²-dG-BPDE-N'11 adduct (N' = CCATCGCTACC), complexed with its natural complementary strand, shows only small perturbations of the B-form double helix with the pyrenyl residue situated in a somewhat widened minor groove [11]. Thus, the thermodynamic and NMR data provide a consistent picture concerning the small perturbations of the duplex caused by trans addition of (+)-BPDE to N² of guanine.

The two methods of evaluating the enthalpy change, from the slopes of $1/T_m$ vs. $\ln C_T$ plots and from the maxima in the $(\partial\alpha/\partial T)_{T=T_m}$ plots, assume that the two-state equilibrium (Eq. 1) is valid. Deviations from this model are indicated if ΔH° is different from ΔH_{VH} [9,12]. In the case of the unmodified duplex, the values of the transition enthalpies derived by these two methods are the same, within experimental error (Table 2). For the modified duplexes, the differences between these two values are significant, suggesting that the melting is not strictly a two-state process and involves different intermediate states [6,9]. A similar observation was made with an anthracen-9-ylmethyl moiety bound to the exocyclic amino group of guanine in a 13-mer oligonucleotide [12].

The mispairing of a BPDE-modified guanine with A, T or G is of interest from the point of view of mechanisms of mutagenesis. The results shown in Fig. 2 (lower T_m) and Table 1 show that the (+)-trans-BPDE-N11 adducts are less stable when duplexed with mismatched bases opposite the modified guanine. However, with T and G opposite the modified G, the transition enthalpy is significantly more negative than in the case of C, the natural complement base. The possibility that at least some of this additional enthalpy is due to pyrenyl-base stacking interactions with opposite mismatched bases was therefore investigated. Evidence from such base stacking interactions is apparent from 1-3 nm small red shifts in the absorption maxima and the hypochromic effects upon duplex formation with mismatched near-complementary strands. In contrast, in the case of the complementary strand with C opposite the modified G, there is a 4 nm blue shift and a hyperchromic effect upon duplex formation (Fig. 4). The latter is consistent with partial pyrenyl-base-stacking interactions in the single-stranded (+)-trans-BPDE-N11 adducts, and a partially solvent exposed minor groove conformation in the duplex as evidenced by fluorescence and CD measurements [3], and NMR structural data [11]. The (+)-BPDE

cis adducts on the other hand, undergo a red shift in the absorption spectrum upon duplex formation with the natural complement, suggesting that there are significant pyrenyl-base interactions in this case in the duplex as well as in the single strand.

In summary, the thermodynamic characteristics of duplexes modified with (+)-BPDE and (-)-BPDE are quite different from one another. How these differences manifest themselves in the biological activities of these stereoisomers remains to be elucidated

II. ORIENTATION OF COVALENTLY BOUND BPDE RESIDUES RELATIVE TO THE DNA 5' → 3' STRAND POLARITY.

INTRODUCTION

The (+)- and (-)-enantiomers of anti-BPDE bind predominantly to the exocyclic amino group of guanine in native DNA, by both trans and cis addition as defined in section I. The high tumorigenicity of the (+)-enantiomer and the low activity of the (-)-stereoisomer are presently not understood. Furthermore, (+)-BPDE is more mutagenic in mammalian cells than (-)-BPDE, but this order of relative mutagenicities is reversed in bacterial cells [1]. Apparently the processing of these BPDE-DNA lesions by various replication and/or repair enzymes is quite different in mammalian and bacterial cell systems. Based on computer modeling studies [13], we predicted that the (+)-BPDE moiety bound trans to the N² position of guanine is (Fig. 5) positioned in the minor groove with the pyrenyl ring system pointing towards the 5'-end of the modified strand, while the pyrenyl ring system of the covalently attached (-)-BPDE enantiomer is pointing towards the 3', or opposite direction. These orientations are depicted in Fig. 5, in which the adducts are embedded in an alternating (dG-dC)₁₂ sequence. These different orientations are a consequence of the chirality of the BPDE stereoisomers. As mentioned in section I, 1-D and 2-D NMR studies have shown that in the case of the (+)-trans-BPDE-N²-dG adduct in the oligonucleotide duplex d(CCATCG^{BPDE}CTACC)·d(GGTAGCGATGG), the pyrenyl residue is indeed pointing towards the 5' direction of the modified strand [11].

We are attempting to develop sensitive methods for determining the relative orientations of aromatic ring systems of polynuclear aromatic diol epoxides covalently bound to nucleic acids. NMR techniques are the methods of choice, but require large amounts of material for study (milligrams); such large amounts of modified oligonucleotide complexes have only recently become available [2]. In most cases, however, only small amounts of polynuclear aromatic diol epoxide-oligonucleotide adducts can be synthesized. We are therefore developing other, more sensitive spectroscopic methods for studying base sequence effects in carcinogen-oligonucleotide adducts. We report here some preliminary results.

Principles of the Methods.

The fluorescence, UV absorption and CD spectra of the aromatic pyrenyl residues are particularly sensitive to their immediate environment. In particular, the fluorescence characteristics can be significantly perturbed by interactions of the pyrenyl residues with neighboring DNA bases. These perturbations can be visualized by comparing the fluorescence characteristics, for example, of dG^{BPDE} mononucleoside adducts, dG^{BPDE}C dinucleotide, and dCG^{BPDE}C trinucleotide adducts. Instead of the cytidines flanking the modified G, any other base could be used. In the dG^{BPDE} adduct case, there is considerable conformational flexibility around the N²-dG-C10BPDE bond, and around the guanine C-N² bond. However, when neighboring bases are present, this conformational flexibility is decreased due to interactions between the base and the bulky pyrenyl residues. These interactions may be sequence-dependent as illustrated in Fig. 6. We expect that even in a dinucleoside, the BPDE residue will be pointing into the 5' direction (Fig. 6, left), while (-)-BPDE will be pointing in the opposite direction (Fig. 6, right). The prediction is that in the modified dinucleoside 5'-dG^{BPDE}C-3' interactions will be minimal, while

(+)-*trans*-BPDE-dG

(-)-*trans*-BPDE-dG

Fig. 5: Minor groove conformations of BPDE-dG adducts in the 12-mer d(GC)₁₂. After ref. 13. The detailed structures of the BPDE-dG lesions are displayed on the top.

in the 5'-dCG^{BPDE}-3' case these interactions will be large. In the case of the (-)-BPDE adducts in the same two sequences, the opposite effects should be observed (Fig. 6).

Fluorescence as a Probe of Pyrenyl-Nearest Neighbor DNA Base Interactions.

We have found earlier [14] that the fluorescence yield and the fluorescence spectra of pyrenyl residues are strongly dependent on the polarity of the environment. Here, we focus on the shape of the fluorescence emission spectra of pyrene-like molecules which are characterized by two prominent vibronic bands at about 379 and 399 nm. The ratio R is defined as the fluorescence intensity (I) peak ratio $R = I_{379}/I_{399}$. This ratio decreases as a function of decreasing polarity of the solvent environment. This effect is illustrated in Fig. 7 in the case of the tetraol BPT (7,8,9,10-tetrahydroxytetrahydrobenzo[a]pyrene), the hydrolysis product of the diol epoxide BPDE.

Fluorescence Intensity Ratios in BPDE-modified d(GC) Sequences of Different Strand Polarities are sensitive to the BPDE Orientation.

A typical example of fluorescence spectra of two different BPDE-dinucleoside adducts are shown in Fig. 8. The fluorescence excitation spectra (240-360 nm) resemble the absorption spectra; the maxima occur at the same wavelengths, but their relative heights are not the same as those in the absorption spectra because we have not corrected the excitation spectra for variations in the output of the light source as a function of wavelength.

The fluorescence emission spectra are very different for the 5'-d(G^{BPDE}C)-3' and the 5'-d(CG^{BPDE})-3' cases. In the former case the R -ratio is ≈ 1.02 , while in the latter case it is only 0.79. We conclude that in the 5'-d(CG^{BPDE})-3' adduct, the pyrenyl residue is located in a less polar environment because it is interacting with the neighboring C as shown in Fig. 6 (left, bottom structure). In the case of the d(G^{BPDE}C)-3' dinucleoside adduct on the other hand, the R -ratio is larger, and the pyrenyl residue is thus located in a more aqueous environment. These characteristics are consistent with the other structure for the (+)-BPDE adduct (Fig. 6, left, top), in which the BPDE moiety points away from the cytosine residue.

In the case of the (-)-BPDE dinucleoside adducts, exactly the reverse effects are observed (data not shown). The R -ratios are summarized in Table 4.

Analogous effects are also observed in the circular dichroism spectra of these same BPDE-dinucleoside adducts (data not shown).

Conclusions

These simple spectroscopic methods appear to be suitable for determining the relative orientations of polynuclear aromatic mutagens in mini-DNA sequences, relative to the 5'---> 3' strand polarity. The results obtained with (+)-BPDE and (-)-BPDE bound covalently, by trans addition, to the exocyclic amino group of guanine, are in accord with theoretical predictions [13], and with NMR structural data in the case of (+)-BPDE. Other research in progress in our laboratory shows

(+)-BPDE

(-)-BPDE

Fig. 6: Predicted orientations of BPDE residues relative to 5'--3' ministrand polarity

Fig. 7:

% H₂O (V/V)

R ratios of fluorescence intensity bands $R = F_{379}/F_{399}$ of BPT in ethylene glycol/aqueous buffer solution of different compositions (2.4 μ M BPT, excitation 343 nm, room temperature).

(S. K. Kim & N. E. Geacintov et al. (1989) Fluorescence spectroscopy of benzo[α]pyrene diol epoxide-DNA adducts. conformation-specific emission spectra. *Photochem. Photobiol.* 50, 327-337)

• The fluorescence spectra of (+)-*trans*-BPDE-GC (top) and (+)-*trans*-BPDE-CG (bottom) at room temperature

Fig. 8.

TABLE 4: Fluorescence R-ratios in BPDE-dinucleoside adducts.

Adducts	R ratio (F_{379}/F_{399})	
	(+)- <i>trans</i>	(-)- <i>trans</i>
5'-G-C-3'	1.02	0.81
5'-C-G-3'	0.79	1.28
BPT in the buffer:		1.12

strong differences in the rates of digestion of (+)- and (-)-BPDE-oligonucleotide adducts by exonucleases (enzymes which break down DNA by hydrolyzing nucleotides starting at the 5' or the 3' end, depending on the enzyme. Apparently, the bulky BPDE residues interfere with the normal functioning of these enzymes, the degree of interference depending on the conformation of the BPDE residues, or perhaps on the orientations of the aromatic pyrenyl residues with respect to the 5'----> 3' direction in DNA. These results, only in their initial stages at this time, are showing great promise for relating chemical structural adduct characteristics with biochemical, and ultimately with their biological characteristics.

Program for the Next Year.

The thermodynamic studies will be continued with sequences for which NMR structural data is becoming available. This includes the sequence d(CCATCG^{BPDE}CTA-CC) in a duplex with its natural complement and with near-complementary strands with mismatched bases opposite the modified dG. These combined thermodynamic and NMR structural studies should provide new insight into the mispairing of bases with BPDE-modified guanines, a problem which is of importance for the understanding of the mechanisms of mutagenesis.

Our exciting results on sequence-effects (described in section II) will be pursued. We have used the sequences d(CG) and d(GC) so far. We believe that the steric hindrance results will be more pronounced if we substitute the bulkier adenine for cytosine and study the sequences d(GA) and d(AG). Subsequently, similar spectroscopic and structural data will be obtained with trinucleotides d(XGY), where the bases X and Y can be any of the four bases.

In a new approach for our laboratory, we will use NMR techniques as well to study the structural characteristics of these mon-, di-, and tri-nucleotide BPDE adducts using our two in-house NMR machines (300 MHz).

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