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MECHANISMS OF INTERACTION OF RADIATION WITH MATTER

PROGRESS REPORT

Period: July 1, 1992 - June 30, 1993

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ABSTRACT

The combustion of fossil fuels give rise to toxic byproducts which pollute our environment and represent potential hazards to human health. Ionizing radiation from nuclear power plants, radioactive waste dumps, and radon in homes pose yet other environmental health hazards. This project is concerned with the mechanisms by which polynuclear aromatic (PNA) compounds on the one hand, and ionizing radiation on the other, cause damage to DNA, the genetic material of the cell. The PNA compounds constitute an important class of environmental pollutants derived from energy-related sources which, upon metabolic activation to diol epoxide derivatives, bind covalently to DNA. These bulky PNA-DNA lesions interfere with the normal DNA replication and transcription processes, and give rise to mutations and the initiation of tumors. Chiral and other stereochemical effects play a key role in determining the biological effects of a given PNA diol epoxide and the potentially mutagenic lesions which are formed. In this project, important breakthroughs have been made by devising new and efficient methods for synthesizing stereochemically pure and precisely positioned PNA diol epoxide-DNA lesions in small DNA fragments (oligonucleotides). In conjunction with several key collaborators, we have elucidated, for the first time, the structures of three stereoisomeric benzo[a]pyrene diol epoxide-DNA adducts. The manifestations of these adducts on DNA polymerase fidelity, transcription, and DNA repair are currently being investigated for detailed structure-biological activity correlations. Spectroscopic techniques such as circular dichroism, fluorescence, and photoionization play an important role in the characterizations of the PNA adducts. In this connection, a new method was developed for measuring the lifetimes as well as the energies of short-lived (picosecond duration) electronically excited states (a patent application has been filed). Using this technique, it is proposed that short-lived (15 ps) charge-transfer (CT) states in the PNA compound tetracene are activated by a short (20 ps) laser pulse; an unusual external photoemission echo due to the recombination of CT states is observed 85 ps after the pulse. The results obtained by this method are relevant to an understanding of the mechanisms of interaction of ionizing radiation with matter.

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INTRODUCTION

Rationale of Project

The production of energy in nuclear power plants or by the combustion of fossil fuels poses a serious potential hazard to human health and the environment. The interaction of ionizing radiation with biological systems, especially cellular DNA, has long been of great concern. The combustion of fossil fuels gives rise to environmental pollutants, including airborne particulates containing deposits of radionuclides and of **polynuclear aromatic (PNA)** derivatives, many of which are potent mutagens and tumorigens.

This project is concerned with the **mechanisms by which these byproducts of modern energy-producing technologies damage DNA**, the genetic material of the cell. Such information may ultimately be important for addressing the adverse health effects associated with these environmental pollutants. Of special interest are **structure/function/activity relationships** which can provide insight into the fundamental mechanisms of these important chemical, physical and biophysical processes.

Studies of **biological activity-structure relationships** are approached from different points of view, recognizing that both the structure of the DNA as well as the structure and characteristics of the PNA compounds are of critical importance in determining the endpoints of their interactions. A basic theme throughout this work is the use of the photoexcited states of PNA compounds as probes of these interactions. A unique feature of our approach is the characterization of the mechanisms of energy dissipation, photoionization, photoinduced electron transfer, and fluorescence of a few key PNA model compounds, and the applications of these phenomena to studies of PNA-DNA interactions. Within the last two years, our repertoire of techniques has been extended to one- and **two-dimensional NMR, high resolution gel electrophoresis, and the influence of PNA-DNA adducts on enzyme activities and function.**

CURRENT RESEARCH GOALS AND SUMMARY OF KEY PROGRESS

I. Structure-biological function relationships in polynuclear aromatic diolepoxide-DNA adducts.

While most PNA compounds are generally inert and unreactive, in living cells they are metabolized to highly reactive and unstable intermediates which are known to react and bind with the genetic material of the cell (1). Within the last year, we have focused our efforts on the biologically active diolepoxide derivatives of three very important PNA compounds: benzo[a]pyrene, 5-methylchrysene, and benzo[c]phenanthrene. The structures of the respective diolepoxides, termed BPDE, 5-MeCDE, and BcPhDE, respectively, are shown in Fig. 1. Our success in synthesizing large quantities of well-defined benzo[a]pyrene diolepoxide-DNA mini-helices for NMR structural studies, and the biochemical characterizations of the properties of these carcinogen- and mutagen-DNA adduct model systems, have led to new advances in understanding how these bulky lesions affect the functioning of several important enzymes. These results are extremely promising, and have opened new lines of research which will lead to novel insights relating the structural characteristics of polynuclear aromatic mutagen-DNA adducts to their toxic biological effects.

II. Synthesis of model DNA adducts.

Our work within the last year has focused on the synthesis of diverse PNA diolepoxide-oligonucleotide adducts, based on modifications of procedures previously published by us (2,3). We selected BPDE, 5-MeCDE, and BcPhDE for detailed studies for the following reasons:

BPDE: the widely studied enantiomers (+)-*anti*-BPDE and (-)-*anti*-BPDE are characterized by strikingly different mutagenicities and tumorigenic activities. Therefore, these two stereoisomers provide a clear example of chiral effects on biological activities of PNA compounds. The major target site of covalent binding of both BPDE isomers to cellular DNA is the exocyclic amino group of guanine.

5-MeCDE: the (+)-*anti*-5-MeCDE and (-)-*anti*-5-MeCDE enantiomers also bind

Fig.1

predominantly to the exocyclic amino group of guanine (N2-dG): However, in contrast to BPDE, in 5-MeCDE the planarity of the aromatic ring system is distorted because of the methyl group at the 5-position. These distortions are often associated with enhanced biological activities (4).

BcPhDE: this is one of the most potent tumorigenic PNA diolepoxides (5). Unlike the other two PNA diolepoxides, BcPhDE binds extensively to the exocyclic amino group (N6) of adenine, as well as to the exocyclic amino group of guanine. The enhanced tumorigenicity of this compound has been associated with two factors: (a) the severe sterical distortions in the crowded bay-region in which the epoxide group is situated (arrow, Fig. 1). , and (2) the experimentally observed strong binding to adenine residues.

An additional complicating factor in the reaction of these PNA diolepoxides with DNA, is that the covalent addition to N2 of guanine and N6 of adenine can proceed by either *cis* or by *trans* addition (defined in Fig. 2).

The types of PNA diolepoxide-oligonucleotide adducts which we have synthesized so far (guanosyl and adenosyl adducts) are summarized in Fig. 3. The oligonucleotide-adducts which have already been subjected to detailed NMR analysis are indicated on the right-hand side of the Figure. Three papers have already been published (papers 8,9, & 16, listed in the NYU bibliography at the end of this report).

Mutations often arise in sequences containing runs of G's. In order to study such biologically important mutation hot spots, we have synthesized BPDE-modified oligonucleotides containing three contiguous G's (bottom of Fig. 3), and the mutation hot spot d(GCG^{BPDE}GCCAAAG) identified recently by Rodriguez and Loechler (6). The synthesis of covalent BPDE-oligonucleotide sequences with several guanine residues present special problems, and some effort was expended within the last year to develop efficient synthetic and purification procedures.

III. Determination of Structures by NMR and Energy Minimization Computation

Methods.

The development of methods for elucidating the conformations of PNA diolepoxide-DNA adducts in aqueous solutions is a top priority of our research program. The structures

Fig. 2

Examples of PAH diolepoxide-oligonucleotide complexes synthesized

11- 16-mers: -----G*----- *BPDE, NMR
 *5-MeChry NMR
 *BPDE Polymerase primer ext.

11-mers: -----A*----- *BcPhDE NMR
 *BPDE

Mutation hot spots in multiple G sequences:

d(GCG*GCCAAAG) *BPDE

Triple G sequences:

d(CTATGGGTATC) -G*GG-, -GG*G-, -GGG*.

Fig. 3

of these lesions are of great significance for an understanding of the mechanisms by which PNA metabolites induce mutations in living systems. This complex effort is a three-way collaboration:

(1) PNA diepoxide-oligonucleotide adduct synthesis at NYU (our laboratory). In this effort, up to 10 mg of stereospecific and site-specific PNA diepoxide-DNA adducts are synthesized and purified. These large amounts are needed for the successful analysis of 2-D NMR data, and represent a substantial amount of work.

(2) The NMR experiments are being carried out by our collaborators M. Cosman (a former graduate student in our laboratory) and D. Patel at the Memorial Sloan-Kettering Cancer Center in New York City. In this work, a combination of exchangeable 1D and 2D proton spectra, and 2D NOESY and COSY techniques, are being used to identify each proton resonance, and to obtain a set of interproton distances.

(3) Energy-minimization techniques based on the program DUPLEX are then used, in conjunction with the proton-proton distances (deduced from the NMR 2D NOESY data sets), as constraints. This phase of the project is being conducted by S. Broyde (Department of Biology, NYU) and B.E. Hingerty (ONRL, Oak Ridge, TN).

The results of these efforts are computer-generated models of the covalent PNA diepoxide-DNA fragments (11 base-pairs long) which are based on the experimental NMR proton-proton distance constraints, and which are consistent with the torsional angle constraints and interatomic repulsive and attractive electrostatic potentials inherent in DUPLEX.

The conformations of four different adducts have been elucidated so far:

(1) (+)-*trans*-N2-BPDE-dG stereochemical isomer in the modified sequence

d(CCATCG^{BPDE}CATCC), described in Paper No. 8 (NYU Bibliography, end of this report).

(2) (-)-*trans*-N2-BPDE-dG stereochemical isomer in the modified sequence

d(CCATCG^{BPDE}CATCC).

(3) (+)-*cis*-N2-BPDE-dG stereochemical isomer in the modified sequence

d(CCATCG^{BPDE}CATCC).

(4) (+)-*trans*-BcPhDE-dA stereochemical isomer in the modified sequence

d(CTCTCA^{BPDE}CTTCC); the manuscript is in preparation.

We now briefly summarize the salient features of these structures.

- (1) **(+)-*trans*-BPDE-N2-dG oligonucleotide adduct.** The pyrenyl ring system is positioned in the minor groove, is directed towards the 5'-end of the modified strand, and stacks over the neighboring GA sugar phosphate backbone of the unmodified complementary strand. The other face of the pyrenyl ring system is exposed to the aqueous solvent. The minor groove widens at the adduct site in order to accommodate the BPDE molecule. All 11 base-pairs are hydrogen-bonded and thus remain in the Watson-Crick B-DNA form.
- (2) **(-)-*trans*-BPDE-N2-dG oligonucleotide adduct.** The structure is very similar to that derived from the chiral (+)-BPDE isomer, except that the pyrenyl ring system points into the opposite direction, the 3'-direction of the modified strand. This adduct is also characterized by a minor groove conformation.
- (3) **(+)-*cis*-BPDE-N2-dG oligonucleotide adduct.** The pyrenyl ring system intercalates into the DNA helix, spanning both the major and the minor grooves. The base-pairing at the site of the BPDE binding is disrupted; the remaining 10 base-pairs are Watson-Crick hydrogen-bonded. Interestingly, the modified guanine is displaced into the minor groove, is perpendicular to the DNA base pair alignment, and stacks over the sugar and cytosine on its 5'-side. Its partner base, a cytosine residue, is displaced into the major groove.
- (4) **(+)-*trans*-BcPhDE-N2-dA oligonucleotide adduct.** Unlike all of the other three guanine adducts, this adenine adduct has a nearly classical intercalative conformation: The BcPhDE-aromatic ring system is located between two neighboring base-pairs, causing a widening of the duplex at this site, with an otherwise minimum disruption of the B-form double helix.

The BPDE-dG adduct modified oligonucleotide structures are summarized in Fig. 4. It is evident that one and the same molecule, BPDE, can give rise to strikingly different adduct conformations! These structure determinations are the first of their kind, and represent the first step in gaining a detailed understanding of how bulky PNA metabolites alter the structure of DNA, and induce mutations. The possibility of investigating the interactions of these

stereospecific and site-specific PNA-DNA adducts with selected enzyme systems is a most exciting prospect which we are just now beginning to exploit.

IV. Influence of BPDE-dG Adduct Conformations on Enzyme Activities and Function.

As noted earlier, the two chiral BPDE isomers (Fig. 1) are characterized by strikingly different biological activities. Our findings that the major (+)-*trans*- and (-)-*trans*-BPDE-N2-dG adducts have similar minor groove conformations, but that they point in opposite directions relative to the intrinsic DNA 5'---->3' strand polarity (Fig. 4), provides a fertile framework for explaining the differences in the biological activities of these stereoisomers. For example, the reversed orders of mutagenicities observed in bacterial and in mammalian cells (1,7,8) may be due to differences in the recognition of these two types of lesions by repair enzymes and/or the cellular replication machinery.

In order to further explore the relationships between biological activity and adduct conformation, we have investigated two different types of enzyme activities: (1) digestion of single-stranded (ss) modified oligonucleotides by exonucleases, and (2) the functioning of a DNA replication enzyme, Pol I (Klenow *exo*⁻ fragment). These topics are the subject of two manuscripts (NYU Bibliography, papers No. 18 and 20, respectively).

1. Enzyme digestion kinetics of stereochemically different BPDE-DNA Adducts.

Exonucleases are enzymes which digest single-stranded DNA from either the 3'-direction (snake venom phosphodiesterase II, or SVPD), or the 5'-direction (spleen phosphodiesterase I, SPD); in both cases a free sugar 5'-OH group, or a 3'-OH group is required, respectively. We have found that the rates of oligonucleotide digestion are markedly slowed when BPDE-dG lesions are present. In other words, the enzymes are stalled at or near the sites of the adducts, hindering normal enzymatic DNA hydrolysis. Allowing sufficiently long incubation times, or increasing the enzyme concentration, leads eventually to full digestion of the single-stranded DNA fragments (full digestion of the oligonucleotides to their mononucleotides). The functioning of these directional enzymes is depicted in Fig. 5.

Interestingly, the rate of digestion depends strongly on the stereochemical properties of the BPDE-dG lesions in the oligonucleotide

Fig. 4

STEREOSELECTIVE EXONUCLEASE ENZYME DIGESTION AND POSITIONS OF STALL SITES

Fig. 5

The (+)-BPDE-dG-modified oligo is much more easily digested by SVPD I (3'----->5' direction of digestion). On the other hand, the relative rates of digestion are exactly reversed when the other enzyme, SPD II (5'---->3' digestion) is employed! In this case, the (+)-BPDE-dG adduct is much more resistant to digestion than the (-)-BPDE-dG-modified oligonucleotide. These differences are depicted in Fig. 5.

Most interestingly, we have investigated the nature of the fragments which are resistant to digestion, and which thus represent the enzyme "stall sites". This was accomplished by high resolution gel electrophoresis experiments in which the length of the fragments causing the "stalling" were compared to standard BPDE-oligonucleotide fragments synthesized especially for this purpose. The location of the stall sites are shown by the dashed bars in Fig. 5. The adduct orientations relative to the 5'-----3' strand polarity are also shown, assuming that the NMR data obtained with modified duplexes also applies to the orientation of the BPDE residues in single strands as well (while this is an assumption, we believe that the locations of the stall sites shown in Fig. 5 fully support this model). Whenever, one or the other enzyme meets an adduct "head on", i.e. the pyrenyl residue points in a direction opposing the normal progression of the enzyme, the latter stalls more easily than when the adduct points in the opposite direction. Also, in the head-on case, the enzyme stalls one base before the adduct. When the adduct points in the same direction as the normal progression of the enzyme, the latter stalls exactly at the modified base!

These findings represent the first known example of the stereoselective inhibition of a pair of enzymes by two stereochemically different bulky PNA carcinogen-DNA adducts which are derived from a pair of chiral BPDE dilepoxide isomers. Furthermore, from studies of the lengths of the DNA fragments representing the stall sites, it is possible to determine the relative adduct orientations in single-stranded DNA. Normally, because of the high flexibility of ss DNA, it is difficult to obtain reliable NMR data with denatured or ss oligonucleotides. Because at DNA replication forks one part of the strand is in the single-stranded form, it is important to study the conformations of adducts in these contexts (NMR experiments in progress).

These startling observations, though obtained with simple exonucleases, suggests why the biological activities of the *trans*-adducts derived from the enantiomers (+)-*anti*-BPDE and (-)-*anti*-BPDE may be different from one another: the bulky pyrenyl residues are oriented in different directions relative to the DNA 5'----->3' strand polarity, thus affecting the functioning of direction-sensitive enzymes to different extents. Based on our findings, it is tempting to speculate that analogous directional effects are of critical importance in the replication past a bulky PNA-DNA lesion *in vivo*. Since polymerase complexes are different in mammalian and in bacterial cell systems, it is possible that the differences in the mutagenicities of (+)-*anti*-BPDE and (-)-*anti*-BPDE may arise from the type of orientational effects observed by us with simple exonucleases.

2. Effects of BPDE-dG Oligonucleotide Adducts on the Functioning of a DNA Polymerase.

DNA polymerases represent another class of enzymes which function directionally with respect to the 5'----3' DNA strand polarity. DNA polymerases use one strand of DNA as a template, and reproduce the complementary strand with high fidelity from the 3' to the 5'-direction. It has been known for a long time that BPDE-DNA adducts block the polymerases partially or completely at or near the adduct sites. Also, from *in vivo* mutagenesis experiments, it is known that incorrect bases can be inserted opposite the BPDE-modified base, or that one or two bases can be missed altogether in the duplicated strand (one- or two-base deletions, denoted by Δ^1 and Δ^2 , respectively). In order to study the effects of the stereochemical properties of BPDE-dG lesions separately, we prepared adducts containing either:

(+)-*trans*-BPDE-dG, (-)-*trans*-BPDE-dG, (+)-*cis*-BPDE-dG, and (-)-*cis*-BPDE-dG adduct lesions in the template oligonucleotide strand:

The strand on the top is the primer strand, while the bottom strand is the template strand which is used to "extend" the primer strand by the polymerase from the 5' end of the primer to the 3'-end. In these experiments, done in collaboration with S. Shibutani and A.P. Grollman (SUNY Stony Brook, NY), the *Escherichia coli* Polymerase Pol I (Klenow fragment without exonuclease editing activity) was used in typical "primer extension" or DNA synthesis experiments catalyzed by Pol I. The nature of the newly synthesized strands ("extended" primer strands) was analyzed by Maxam,-Gilbert gel sequencing methods. In this way, the nature of the deletions or base-substitutions (mutations) can be determined.

The results are summarized in Table I. The only base that is incorrectly substituted is adenine in all cases. However, the efficiency depends strongly on the stereochemistry of the BPDE-dG adducts, the (-)-*trans*-isomer being apparently most mutagenic in this system. The highest fractions of deletions are observed in the case of the (-)-*cis* adducts, as shown in Table I. The ramifications of these results are fully discussed in paper No. 20 (NYU bibliography).

These are also initial results which prove, once again, how the functioning of enzymes can be affected differently by stereochemically distinct BPDE-dG adducts. However, we believe that the apparent mutation spectra will depend on the nature of the DNA polymerase used, the base sequence in which the adduct is embedded, and the presence or absence of editing exonucleases. These variables represent immensely rich possibilities for studying carcinogen-DNA adduct structure-biological function relationships in future years.

V. Fundamental, Physico-Chemical Studies of Interactions of Radiation with PNA Compounds and with DNA.

In this report, only short summaries of these aspects of our research is provided here, and full progress reports are planned for next year.

1. Mechanisms of Non-Linear Photoionization Processes in PNA Solids and Biological Materials: Time-Resolved Double-Pulse Picosecond Laser Studies.

During the last year a new, original and general method was developed for measuring the lifetimes of short-lived excited states in the condensed phase (9, and manuscript C in the

TABLE I

PRIMER EXTENSION IN REACTIONS CATALYZED BY EXO-KLENOW
FRAGMENT
(E. coli POL I) ON TEMPLATES CONTAINING STEREOISOMERIC BPDE-N2-dG
LESIONS.

TEMPLATE: 5'-CCTTCG^{BPDE}CTCCTTTCCTCT

MISINCORPORATION OF dA OR ONE- (Δ^1) OR TWO-BASE DELETIONS (Δ^2)			
LESION	dA	Δ^1	Δ^2
(+)- <i>trans</i> -BPDE	0.19±0.02%	1.66±0.07%	0.49±0.06%
(-)- <i>trans</i> -BPDE	9.12±0.05%	1.43±0.03%	0.97±0.07%
(+)- <i>cis</i> -BPDE	0.43±0.06%	0.31±0.01%	0.66±0.05%
(-)- <i>cis</i> -BPDE	0.86±0.07%	9.88±0.24%	5.42±0.73%

Conditions: 120 min reaction time, 10 units of exo⁻ Klenow fragment, 25 °C.

EXPERIMENTS BY S. SHIBUTANI, SUNY STONY BROOK

(S. Shibutani, L.A. Margulis, N.E. Geacintov and A.P. Grollman, Biochemistry, in press).

appendix). This method is based on a multi step-emission process in which the decay of an excited state generated by one pulse is measured by means of the photoemission produced by the photoionization of the excited state by means of a delayed probe laser pulse. The 20 ps duration, 355 nm laser pulses were generated by a YAG laser, and the PNA compound tetracene was selected for study. The apparatus in which the measurements were made is a variation of the Millikan apparatus for determining the charge of the electron (10), and the same apparatus and method can be used to measure the durations of the light pulses in the femtosecond time range. A patent application covering this invention has already been filed by the University.

As is discussed in Appendix C, a charge-transfer state of tetracene of effective lifetime 16 ps was identified. Charge transfer excitons are important intermediate states in most ionization and carrier recombination processes, so their properties are vital to understanding these processes.

The results were calculated by analyzing the photoemission yield as a function of the delay between the first and second laser pulses, as is shown in Fig. 1 of Appendix C. two notable features characterize Fig. 1, in addition to the main peak from which the effective lifetime of the CT exciton was deduced. These are the appearance of a small peak in the photoemission yield at -125 ps, and the absence of a similar peak at +125 ps, where it should have appeared because of the asymmetry in the experimental apparatus. As is described in appendix D, the asymmetry was caused by the inequality of light intensities of the first and second pulses (Fig. 2, Appendix D), and the small peak is identified as an "echo" of the CT population in the first (main) peak of Fig. 1 (Appendix A). This CT echo is produced by the dissociation of CT excitons by singlet excitons. Using a kinetic scheme that incorporates this additional process, the experimental data can be completely rationalized, as is shown in Fig. 3 of Appendix D. The existence of a CT echo exposes the ability of a CT exciton to catalytically quench singlet excitons. Thus, a singlet exciton is quenched in the process of dissociating into a hole and an electron, but the hole and electron must recombine to regenerate a CT exciton, which can repeat the quenching process. The quenching of electronically excited states inside a condensed phase that has been subjected to strongly ionizing radiation is well-known as ionization quenching. We propose that the CT exciton

contributes to this quenching process.

2. Fundamental Characteristics and Physicochemical Characteristics of PNA Compounds.

Photophysical investigation of photoionization, photoinduced electron transfer, and fluorescence mechanisms in PNA-DNA complexes. These properties act as highly useful probes of the interactions of the bulky PNA aromatic residues with adjacent nucleic acids. The results of this work have been published or will be shortly published (NYU Bibliography, papers 5,7,10,12,15, and 21).

3. Structural Effects in DNA Damage Induced by Ionizing Radiation.

The mechanisms and patterns of damage produced by different types of ionizing radiation (neutrons and gammas) and chemical agents (hydroxyl radicals) are being investigated using short DNA segments (deoxyoligonucleotides) containing specialized structures often encountered *in vivo* (loops, hairpins, Holliday junctions). This area of research has been relatively neglected and little is known about the effects of ionizing radiation when DNA is not in its usual single-stranded, and double-stranded Watson-Crick form. High resolution gel electrophoresis methods are employed in this work (paper No. 17).

REFERENCES

1. Conney, A.H. (1982) *Cancer Res.* **42**, 4875-4917.
2. Cosman, M., Ibanez, V., Geacintov, N.E. and Harvey, R.G. (1990) *Carcinogenesis* **11**, 1667-1672.
3. Geacintov, N.E., Cosman, M., Mao, B., Alfano, A., Ibanez, V. and Harvey, R.G. (1991) *Carcinogenesis* **12**, 2099-2108.
4. Hecht, S.S., Amin, S., Huic, K., Melikian, A.A. and Harvey, R.G. (1987) *Cancer Res.* **47**, 5310-5315.
5. Agarwal, S.K., Sayer, J.M., Yeh, H.J.C., Pannell, L.K., Hilton, B.D., Pigott, M.A., Dipple, A., Yagi, H. and Jerina, D.M. (1987) *J. Am. Chem. Soc.* **109**, 2497-2504.
6. Rodriguez, H. and Koechler, E.L. (1993) *Biochemistry* **32**, 1759-1769.
7. Stevens, C.W., N. Bouck, Burgess, J.A., and Fahl, W.E. (1985) *Mutat. Res.* **152**, 5-14.

8. Brookes, P. and Osborne, M.R. (1982) *Carcinogenesis* **3**, 1223 - 1226.
9. Durandin, A., Liu, T.-M., Pope, M., Sheu, S.-C., and Geacintov, N.E., *J. Phys. Chem.* **97**, 2-4 (1993).
10. Altwegg, L., Pope, M., Arnold, S., Fowlkes, W.Y., and Hamamsy, M.A., *Rev. Sci. Instrum.* **53**, 332-337 (1982).

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(July 1, 1991 - June 30, 1993)

1. "Applications of luminescence techniques in studies of polycyclic carcinogen-nucleic acid interactions." N.E. Geacintov and S.K. Kim, in: *Practical Spectroscopy Series, Vol. 12: Luminescence Techniques in Chemical and Biochemical Analysis*. (W.R.G. Baeyens, D.D. Keukeleire, and K.K. Korkidis), Marcel Dekker, Inc., New York, pp. 317-339 (1991).
2. "Structures of the (+) and (-)-trans-anti-BPDE adducts to guanine-N2 in a duplex dodecamer." S.B. Singh, B.E. Hingerty, U.C. Singh, J.P. Greenberg, N.E. Geacintov and S. Broyde, *Cancer Res.*, (1991) **51**, 3482-3492.
3. "Chemical, molecular biology, and genetic techniques for correlating DNA base damage induced by ionizing radiation with biological end-points." N.E. Geacintov and C.E. Swenberg, in: *Physical and Chemical Mechanisms in Molecular Radiation Biology*, M.N. Varma and W.A. Glass, Eds., Plenum Press, pp. 453-474 (1991).
4. "Characterization of the interaction of the radioprotectant 1-methyl-2-[2-(methylthio)-2-piperidinovinyl]-quinolinium iodide with supercoiled DNA." C.E. Swenberg, S. Birke, and N.E. Geacintov, *Radiation Research*. **127**, 138-145.
5. "Photoinduced electron transfer in a covalent benzo[a]pyrene diol epoxide-2'-deoxyguanosine complex." L. Margulis, P.F. Pluzhnikov, B. Mao, V.A. Kuzmin, Y.J. Chang, T.W. Scott and N.E. Geacintov (1991), *Chem. Phys. Lett.* **187**, 597-603.
6. "Dependence of conformations of benzo[a]pyrene diol epoxide-DNA adducts derived from stereoisomers of different tumorigenicities on base sequence." C.J. Roche, A.M. Jeffrey, B. Mao, A. Alfano, S.K. Kim., V. Ibanez and N.E. Geacintov, *Chem. Res. Toxicol.* **4**, (1991), 311-317.
7. "Spectroscopic characteristics and site I/site II classification of cis and trans benzo[a]pyrene diolepoxide enantiomer-guanosine adducts in oligonucleotides and polynucleotides." N.E. Geacintov, M. Cosman, B. Mao, A. Alfano, V. Ibanez and R.G. Harvey (1991) *Carcinogenesis* **12**, 2099-2018.
8. "Solution conformation of the major adduct between the carcinogen (+)-anti-benzo[a]pyrene diol epoxide and DNA." M. Cosman, C. de los Santos, R. Fiala, B.E. Hingerty, S.B. Singh, V. Ibanez, L.A. Margulis, D. Live, N.E. Geacintov, S. Broyde and D.J. Patel (1992) *Proc. Natl. Acad. Sci. (USA)* **89**, 1914-1918.
9. "Influence of benzo[a]pyrene diol epoxide chirality on solution conformations of DNA covalent adducts: the (-)-trans-anti-[BP]G.C adduct structure and comparison with the (+)-

trans-anti-[BP]G.C enantiomer." C. de los Santos, M. Cosman, B.E. Hingerty, V. Ibanez, L.A. Margulis, N.E. Geacintov, S. Broyde and D.J. Patel (1992) *Biochemistry* **31**, 5245-5252.

10. "Photoinduced electron transfer and fluorescence mechanisms in covalently linked polynuclear aromatic nucleotide complexes." N.E. Geacintov, B. Mao, L.L. France, R. Zhao, J. Chen, T.-M. Liu, N.-Q. Ya, L.A. Margulis, and J.C. Sutherland (1992), *S.P.I.E. Proceedings, Time-Resolved Laser Spectroscopy in Biochemistry III*, Vol. **1640**, 774-783.

11. "Direct synthesis and identification of benzo[a]pyrene diol epoxide-deoxyguanosine binding sites in modified oligodeoxynucleotides." B. Mao, L.A. Margulis, B. Li, V. Ibanez, H. Lee, R.G. Harvey and N.E. Geacintov (1992) *Chem. Res. Toxicol.* **5**, 773-778 (1992)..

12. "Identification and quantitative detection of isomeric benzo[a]pyrene diolepoxide-DNA adducts by low-temperature conventional fluorescence methods." R. Zhao, T.-M. Liu, S.K. Kim, M.C. McLeod and N.E. Geacintov (1992) *Carcinogenesis* **13**, 1817-1824.

13. "Differences in unwinding of supercoiled DNA induced by the two enantiomers of anti-benzo[a]pyrene diol epoxide." Rong Xu, Sheryl Birke, Susan E. Carberry, Nicholas E. Geacintov, Charles E. Swenberg, and Ronald G. Harvey. *Nucleic Acids Research.* **23**, 6167-6176 (1992).

14. "Picosecond spectroscopy of excited states based on non-linear electron photoemission." A. Durandin, T.-M. Liu, M. Pope, S.-C. Sheu, and N.E. Geacintov, *J. Phys. Chem.* **97**, 2-4 (1993).

15. "Fluorescence HPLC methods for detecting benzo[a]pyrene-7,8-dihydrodiol 9,10-oxide-deoxyadenosine adducts in enzyme digests of modified DNA: improved sensitivity. J. Chen, M.C. MacLeod, R. Zhao and N.E. Geacintov. *Carcinogenesis* **14**, 1049-1051 (1993).

16. "Solution conformation of the (+)-cis-anti-[BP]dG adduct in a DNA duplex: intercalation of the covalently attached benzo[a]pyrenyl ring into the helix and displacement of the opposing dC on the partner strand." M. Cosman, C. de los Santos, R. Fiala, B. Hingerty, V. Ibanez, E. Luna, R.G. Harvey, N.E. Geacintov, S. Broyde and D.J. Patel, *Biochemistry* **32**, 4145-4155 (1993).

IN PRESS (6/30/93)

17. "High resolution gel electrophoresis methods for studying sequence-dependence of radiation damage and effects of radioprotectants in deoxyoligonucleotides." B. Mao, C.E. Swenberg, Y. Vaishnav and N.E. Geacintov (1992) in: *Biological Effects and Physics of Solar and Galactic Solar Radiation*, Eds. C.E. Swenberg, G. Horneck, and E.G. Stassinopoulos, Proceedings of NATO-ASI Conference, Armacao de Pera, Portugal, October 1991. Plenum

Press, New York, (1993).

18. "Opposite stereoselective resistance to digestion by phosphodiesterases I and II of benzo[a]pyrene diepoxide-modified oligonucleotide adducts." B. Mao, B. Li, S. Amin, M. Cosman and N.E. Geacintov, *Biochemistry*, (1993).
19. "Unwinding and hydrodynamic flow linear dichroism characteristics of supercoiled DNA covalently modified with two isomeric methyl chrysene diepoxides of different biological activities." L. Balasta, R. Xu, N.E. Geacintov, C.E. Swenberg, S. Amin, and S.S. Hecht. *Chem. Res. Toxicol.* (1993).
20. "Translesional synthesis on a DNA template containing a single stereoisomer of dG-(+)- or dG-(-)-anti-BPDE (7,8,-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene)." S. Shibutani, L.A. Margulis, N.E. Geacintov and A.P. Grollman, *Biochemistry* (1993).
21. "Mechanisms of quenching of the fluorescence of a benzo[a]pyrene tetraol metabolite model compound by 2'-deoxynucleosides." Nicholas E. Geacintov, Rushen Zhao, Vladimir A. Kuzmin, Seog K. Kim, and Lorenzo J. Pecora. *Photochem. Photobiol.* (1993).

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