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MOLECULAR UNDERSTANDING OF MUTAGENICITY USING POTENTIAL ENERGY METHODS

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The objective of our work, for many years, has been to elucidate on a molecular level, at atomic resolution, the structures of DNAs modified by highly mutagenic aromatic amines and hydrocarbons. The mutagenic specificity of these compounds has been explored by a number of groups. As controls, we have also studied their less mutagenic chemically related analogs, and unmodified DNAs. The ultimate purpose of this undertaking is to obtain an understanding of the relationship between DNA structure and mutagenicity.

The underlying hypothesis is that DNA replicates with reduced fidelity when its normal right-handed B-structure is altered, and one result is a higher mutation rate. This change in structure may occur normally at a low incidence, for example by the formation of hairpin loops in appropriate sequences, but it may be enhanced greatly after covalent modification by a mutagenic substance. Other mechanisms may also lead to mutation after covalent modification, for example the operation of induced error-prone repair pathways. Ultimately it is DNA structure, mediated by replication and repair enzymes, that determines whether a mutation will take place.

The methods that we use to elucidate structures are computational, but we keep in close contact with experimental developments, and we incorporate data from NMR studies in our calculations when they are available. The reason why computational approaches to structure generation are so important in this area is that x-ray and low resolution spectroscopic studies have not succeeded in producing atomic resolution views of mutagen and carcinogen-oligonucleotide adducts. Even the high resolution NMR method cannot alone yield molecular views, though it does so in combination with our computations.

The specific methods that we employ are minimized potential energy calculations using the torsion angle space molecular mechanics program DUPLEX (developed and written by Brian E. Hingerty) to yield static views. Molecular dynamics simulations of static structures with solvent and salt can be carried out with the program AMBER; this yields mobile views in a medium that mimics aspects of the natural aqueous environment of the cell. We wish to thank the DOE for the generous support of our work, including especially the excellent computing resources afforded to us at the NERSC.

Our collaboration with Nicholas Geacintov, Chemistry Department, NYU and Dinshaw Patel, Memorial Sloan Kettering Cancer Center has been fruitful. Our modeling efforts, in combination with the synthetic breakthrough in the Geacintov laboratory that has permitted preparation of site-specific pure adducts in high yield, together with the high resolution NMR work in the Patel laboratory, has produced atomic resolution solution structures for the following adducts in DNA duplexes: 2-aminofluorene (AF) C8-dG opposite A, (+)-trans-anti-benzo-[a]-pyrene-diol-epoxide (BPDE)-N^2-dG opposite C, (-)-trans-anti-BPDE-N^2-dG opposite C, (+)-cis-anti-BPDE-N^2-dG opposite C, and (+)-trans-anti-benzo[c]phenanthrene-diol-epoxide (BPh)-N^6-dA opposite T. In addition, with Krugh at the University of Rochester, who performed the synthesis and high resolution NMR work, we elucidated the solution structure of 2-acetylaminofluorene (AAF)-C8-dG opposite C in a duplex nonamer. These are the only atomic resolution solution structures of polycyclic aromatic hydrocarbon or amine structures in the literature to date, as far as we are aware. Abstracts of the recent publications and illustrations of the structures from these works are given at the end of this report.

In addition, we are continuing our modeling studies without experimental data. In a recent collaboration with Dezider Grunberger and Adelaide Crothers, Columbia University College of Physicians and Surgeons, and Elizabeth Snyderwine of NIH, we investigated the food pyrolysis product 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PHIP)-C8-dG adduct opposite A in a duplex 7-mer. We performed the modeling and our collaborators carried out experimental work in mutagenicity and repair. Results of this work have been submitted to the Journal of Molecular Biology. The Abstract and illustration of the structure are also given at the end of this report.

In the above structures we investigated double stranded DNA with the modified base partnered normally, as it would be prior to replication, or mismatched to a base that has been experimentally observed to be a prevalent misincorporation event for the given lesion; this would represent the view following unfaithful replication. In addition, we are interested in the view during replication. Here, a single strand-double strand junction is an interesting structure to model as it represents one strand of the replication fork. To begin studying this novel structure we investigated the (+)-trans-anti-BPDE-N^2-dG adduct in the sequence:

parent 5′-d(G1-C2-G3-C4-G5-C6-[BP]G7-C8-G9-C10-G11-C12)-3′
| | | | |
daughter 3′-(G17-C16-G15-C14-G13)

In this sequence [BP] G7-C6-G5 are the next residues to be replicated. The [BP] moiety was positioned in the B-DNA minor groove oriented toward the 5′-end of the modified strand in the conformation that we had predicted (Singh et al. (1991) Cancer Res. 31, 3482-3492) and that had been independently observed by high resolution NMR (Cosman et al. (1992) Proc. Natl. Acad. Sci. USA 89, 1914-1918).

A 500 picosecond molecular dynamics simulation with 3,185 water molecules and 15 Na$^+$ ions was performed. At 383 picoseconds an interesting box-like structure appeared involving the next to be replicated [BP]G7-C6-G5; specifically, C6 rotates, from its normal position stacked with G5 and G7, so that it is stacked with the BP, and this structure persists till the end of the simulation at 500 picoseconds. Such a structure can be envisioned to block replication as has been observed (Hruszkewycz et al. (1992) Carcinogenesis 13, 2347-2352; Shibutani et al. (1993) Biochemistry 32, 7531), to invoke error-prone repair that results in mutations, or to be directly miscoding. The abstract of this manuscript, prepared for DNA Damage; Effects on DNA Structure and Protein Recognition, Annals of the New York Academy of Sciences, and structure illustration are presented at the end of this report. A video of this simulation has been prepared for us by David Butler of the Sandia National Laboratories.

We have continued to explore methods for generating structures for carcinogen-modified DNA fragments by combination of small subunits, without reference to experimental input. This would allow us, for example, to compare the effect of all possible 5' and 3' neighbors adjacent to a carcinogen-modified base in DNA, and to search for a structural basis for observed "hotspots" in mutagenesis and carcinogenesis. The multiple minimum problem has been one formidable problem in such an effort, as a thorough search of conformational space consumes substantial computational time and there is, in any event, no guarantee that the global minimum will be located. We address the problem, however, by a search and build strategy that explores small DNA subunits first and then builds to larger units via a number of strategies we have devised. In our previous report, we presented the results of a search of AF- and AAF-modified dinucleoside monophosphates. These fragments represent the smallest DNA unit above the nucleotide level, yet even with bond lengths and angles held constant, they present 14 torsional variables for AF-modification and 16 for AAF-modification. A search was conducted for 8 AF-modified and 8 AAF-modified dimers, using 4,897 trials in each case. Several provocative stable minima were encountered, with possible implications for mutagenesis. In selected sequences, an additional 31,104 trials were run, but they did not enhance the results substantially.

This set of results has now been used to test an alternative strategy for locating minima: the method of simulated annealing (Kirkpatrick, et al. (1983) Science 220 , 671-680; Cerny (1985) J. Optical Theory & Appl. 45., 41-51). In this approach, the starting conformation and subsequent angle changes are introduced at random. The option exists for temporarily accepting a change which increases the energy of a calculated structure. Such uphill walks on the energy surface]give the SA technique its unique power, for they enable the molecule to climb out of higher energy local minima; this improves the chances of reaching the lower energy wells. In effect, the strategy can traverse the potential energy surface in multi-dimensional conformational space.

The simulated annealing approach was applied independently to the AF- and AAF-modified dinucleoside monophosphates. It located seven new global or co-global minima not found by our previous minimization method (BOTM), and in the remaining cases identified the existing global minima (in some cases the energy was slightly higher or lower due to changes in the terminal angles which did not affect the important features of the conformer). The SA method also identified several low energy types of conformer not located by the earlier method, though it missed a smaller number identified by the other method. It provides a valuable alternative or supplemental tool for exploring the conformation of the smallest DNA fragments. Its applicability to the larger ones awaits future study. A manuscript detailing this work has been submitted and the Abstract is included at the end of this report.

Another strategy has been used to explore the conformation of aromatic amine modified DNA double-stranded trimers. The central nucleotide was placed in a number of orientations which have proved significant in our previous studies: conventional B-DNA, Hoogsteen, wedge and others. The angles that determine the position of the modifying amine were then allowed to vary and stable minima sought, using the BOTM algorithm and our hydrogen-bond forcing function. This method has been applied to a number of modifying amines, AF, AAF, aniline, aminobiphenyl, and aminopyrene, using 16 sequences in each case. Characteristic major and minor groove families have been determined in each case (except for AAF, in which anti-conformations are destabilized), and several novel structural types have been encountered. The most interesting result has emerged from the 4-aminobiphenyl-modified series, in which modification at the 8-position of guanine and of adenine were compared. The geometries of the major groove families were very similar for the adenine and guanine adduct families, but the former was destabilized by 10 kcal/mol when compared to the latter. As a result, a characteristic structural type with the amine in the minor groove provided most of the global minima in the adenine series, while a B-DNA structure with the amine in the major groove served as the global minimum for the majority of sequences in the guanine series. This result may be relevant to the study of aminobiphenyl mutagenesis by Lasko, et. al, (1988, J. Biol. Chem. 263, 15429-15435) in which the hot spot of most frequent occurrence was located at an adenine residue.

Publications

1. "Solution Conformation of the Major Adduct Between the Carcinogen (+)-anti-Benzo[a]pyrene-diol-Epoxide and DNA", Cosman, M., de los Santos, C., Fiala, R., Hingerty, B. E., Singh, S. B., Ibanez, V., Margulis, L., Live, D., Geacintov, N., Broyde, S. and Patel, D. J., Proc. Natl. Acad. Sci. USA 89, 1914-1918 (1992).

2. "Structure of an Acetylaminofluorene (AAF) Modified DNA Oligomer" O'Handley, S. F., Sanford, D. G., Xu, R., Lester, C., Hingerty, B. E., Broyde, S. and Krugh, T. R., in Structure and Function, Volume I, Nucleic Acids, 1992, pp. 137-146, Adenine Press, Schenectady, NY.

3. "Influence of Chirality of Anti-Benzo-[a]-pyrene-diol-Epoxide on Solution Conformations of its DNA Covalent Adducts: The (-)-trans-anti [BP]G.C Adduct Structure and Comparison with the (+)-trans-anti Enantiomer", de los Santos, C., Cosman, M., Hingerty, B. E., Ibanez, V., Margulis, L., Geacintov, N. E., Broyde, S. and Patel D. J., Biochemistry 31, 5245-5252 (1992).

4. "Structural Characterization of an N-acetyl-2-aminofluourene (AAF) Modified DNA Oligomer by NMR, Energy Minimization, and Molecular Dynamics", O'Handley, S. F., Sanford, D. G., Xu, R., Lester, C. C., Hingerty, B. E., Broyde, S., and Krugh, T. R., Biochemistry 32, 2481-2497 (1993).

5. "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 Modified Deoxyguanosine", Cosman, M., de los Santos, C., Fiala, R., Hingerty, B. E., Ibanez, V., Luna, E., Harvey, R., Geacintov, N. E., Broyde, S. and Patel, D. J., Biochemistry 32, 4145-4155 (1993).

6. "Solution Conformation of the (+)-trans-anti-[BPh]dA Adduct Opposite dT in a DNA Duplex: Intercalation of the Covalently Attached benzo[c]phenanthrene to the 5′ side of the Adduct Site without Disruption of the Modified Base Pair", Cosman, M., Fiala, R., Hingerty, B. E., Laryea, A., Lee, H., Harvey, R. G., Amin, S., Geacintov, N. E., Broyde, S. and Patel D. J., Biochemistry, In Press.

7. "Mutation and Repair Induced by the Carcinogen, 2-amino-1-methyl-6-phenyl-imidazo[44,5-b]pyridine in the CHO Dihydrofolate Reductase Gene and Conformational Modeling of the dG-C8-PHIP Adduct in DNA", Crothers, A. M., Yuan, W., Hingerty, B. E., Broyde, S., Grunberger, D. and Snyderwine, E. G., Submitted.

8. "Molecular Dynamics Simulation of a Tumorigenic Benzo[a]pyrene Metabolite Bound to DNA at a Single Strand-Double Strand Junction", Singh, S. B., Li, B., Hingerty, B. E., Shapiro, R. and Broyde, S. in DNA Damage: Effects on DNA Structure and Protein Recognition, Annals of the New York Academy of Science, In Press.

9. "The Torsion Space Nucleic Acids Molecular Mechanics Program DUPLEX: Surveying Conformation Space by Potential Energy Minimization; A Progress Report", Hingerty, B. E. and Broyde, S., Proceedings of the 8th Conversation in Biomolecular Stereodynamics", In Press.

10. "Conformation of Amine Modified DNA: AF and AAF Modified Deoxydinucleoside Monophosphates with all Possible Nearest Neighbours. A Comparison of Search and Optimization Methods", Shapiro, R., Sidawi, D., Miao, Y. -S., Hingerty, B. E., Schmidt, K. E., Moscowitz, J. and Broyde, S., Submitted.

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