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Simulation of Biomolecular Complexes: Advanced Computational Sciences at the Molecular Level

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Abstract

We will use advanced theoretical modeling to study the interactions between bioreceptors (DNA, protein, antibodies) with target species at the molecular level. We will use a unique, advanced computational program, DUPLEX, developed by B. Hingerty (PI) to computationally determine specific structures of RNA, DNA, damaged DNA, and DNA-protein complexes. We will expand our extensive previous experience with carcinogen-DNA adducts to model target bioreceptors. Experimental studies will be done in collaboration with T. Vo-Dinh and his coworkers at ORNL using unique nanosensors for the real-time detection of a wide range of biotargets. These nanosensors will take advantage of near-field spectroscopy to monitor the interactions of various bioreceptor molecules (DNA, antibodies, enzymes, RNA) with their respective analytes at the molecular level. These fundamental experiments, will be used to determine and evaluate the simulation models. NMR data will be collected by M. Cosman at Lawrence Livermore National Laboratory.

Progress Report

Objective and Technical Approach

We will do computational modeling of modified DNA structures from known carcinogens (such as G-POB) as well as DNA-protein complexes utilizing NMR data from solution structures in collaboration with M. Cosman at Lawrence Livermore National Laboratory. The theoretical studies will be done in collaboration with Prof. S. Broyde at New York University.

The tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone(NNK) and N-nitrosonornicotine (NNN) are potent carcinogens in laboratory animals. NNK is carcinogenic to the lung, liver, nasal cavity, and pancreas. NNN is carcinogenic to the esophagus, nasal cavity, and respiratory tract. NNK can be metabolized to produce either a methylating agent or a pyridyloxobutylating agent. An O^6-[4-oxo-4-(3-pyridyl)-butyl]guanine **(G-POB)**, was detected in DNA treated with a directly acting pyridyloxobutylating agent and this same adduct was recently observed in the liver DNA of mice treated with NNK. This adduct has been successfully incorporated into DNA segments by totally synthetic methods, and it exhibited the unexpected ability to serve as a substrate for repair by the recombinant human and rat liver O^6-alkylguanine-DNA alkyltransferase (a DNA repair enzyme). G-POB is a potent mutagenic lesion in DNA and, provided it escapes alkyltransferase repair, it can contribute significantly to the mutagenic risk posed by exposure of human cells to NNN and NNK in cigarette smoke. The chemical isolation of G-POB and its' incorporation into DNA segments was done by Dr. L. Peterson at the Univ. of Minnesota Cancer Center, Mayo Clinic, Minneapolis, MN and the subsequent NMR data was collected by M. Cosman at Lawrence Livermore National laboratory.

Another target structure called **Protamine** is a protein that binds sperm DNA. Its' processing is critical for male fertility. Our goal is the following:

1) Determine the structure of the protamine-DNA complex
2) Understand how protamine competes effectively with other DNA binding proteins
3) Understand why protamine binding prevents damaged DNA from being repaired

Results to Date

G-POB

M. Cosman obtained an 11 base pair structure of DNA modified by G-POB and the NMR solution spectra was collected. This structure is as follows:

C1- C2- A3- T4- A5- T6- G7*- G8- C9 - C10- C11
G22-G21-T20-A19-T18-A17-C16-C15-G14-G13-G12 where G7* is G-POB.

This structure was then analyzed computationally with the program DUPLEX and after several iterations a reasonable model was produced that agrees with the NMR data and had a defined minimum potential energy. The model is currently being optimized on the new massively parallel IBM SP computer at NERSC (National Energy Research Scientific Computing Center) at Lawrence Berkeley National Laboratory.

Protamine

Preliminary work on this project has been started. A good complex has been obtained and we have started to collect NMR data. The analysis is still in progress and further experiments need to be run to obtain the best possible NMR restraint list for DUPLEX. The CYANA program, which does protein structures from NMR data, needs to be purchased and used with DUPLEX in the analysis of this data.

DNA Hybridization Structures

Some preliminary calculations have been done for DNA structures with slipped base pairs and bulges. These structures such as triplet repeats are present in several known genetic diseases such as myotonic dystrophy, Huntington's disease and fragile X-syndrome. These results are being analyzed. T. Vo-Dinh will do recognition of such structures by DNA probes.

Summary and Benefits

We feel these extensive collaborations will be of benefit to ORNL and DOE and may lead to funding from either the NIH or the DOE Genomes to Life Program.

Publications

B. Hingerty, M. Cosman, D. J. Patel and S. Broyde, "Molecular Views of Carcinogen Damaged DNA", Invited Talk at the 7th World Congress on Advances in Oncology and the 5th International Symposium on Molecular Medicine, 10-12 Oct 2002, Hersonissos, Crete, Greece. Session co-chair of the Epidemiology and Carcinogenesis session.