Could you begin with an overview of your research focus? What are the chemical reactions central to your work?

My research focuses on the toxicology and biosensing properties of 2’-deoxyguanosine (dG). Specifically, we examine dG that has been modified on the eighth carbon atom by certain aromatic ring compounds called aryl residues, a focus which stems from earlier research in my laboratory. This demonstrated that toxins based on phenol, an aromatic ring with a hydroxide group, are able to react covalently at the C8 position of dG. The reaction generates both carbon (C)- and oxygen (O)-linked C8-dG adducts. In other words, the carbon and oxygen are able to bond covalently to the dG, creating one molecule from the two.

What role do these reactions play? What are you ultimately hoping to achieve?

During the course of our investigations, we found that the C-linked C8-dG adducts are highly fluorescent, and could consequently be used as biosensors. These nucleic acid polymers frequently bind in a sequence-specific manner to DNA and RNA, usually forming duplexes, and consequently they are useful as probes. The fluorescence we have uncovered would work within this, creating biosensors to study metal ion, small molecule and protein binding by oligonucleotides.

Thus, one of our goals is to characterise the toxicological properties of C- and O-linked adducts and determine how DNA enzymes process these lesions. A second goal is to develop DNA oligonucleotides, called aptamers, which possess fluorescent C8-dG bases and provide an optical signal when the aptamer binds to its molecular target. Such aptamers would have practical applications in medicine and agriculture.

Can you summarise the genetic pathway xenobiotics (foreign substances within an organism) make in attaching to and altering DNA function?

Most xenobiotics that react covalently with DNA undergo bioactivation to generate electrophilic species, which may be electron-deficient or highly strained, short-lived molecules. These, in turn, react with nucleophilic sites on the DNA bases, which are electron rich and therefore attract electrophiles. Enzymes involved in xenobiotic metabolism are numerous, but these enzymes typically convert the xenobiotic into more water soluble derivatives or metabolites that are eliminated in urine and faeces. DNA adducts are formed at very low levels, but if left unrepaired they can cause DNA polymerase enzymes to make errors during DNA replication, generating mutations that can ultimately lead to cancers.

Which synthetic strategies are you utilising in this assay to establish primer extension?

Primer extension assays map the five prime ends of DNA and RNA, and can be used to determine the start site of RNA transcription for a known gene. They require the synthesis of oligonucleotide substrates that are fairly lengthy for the DNA polymerase, the enzyme that reads’ DNA bases, to bind to.

Initially, we developed a post-synthetic strategy to incorporate C8-aryl-dG adducts site-specifically into DNA substrates that relies on an approach called the Suzuki cross-coupling reaction. This method is suitable for incorporating a single C8-aryl-dG adduct into short oligonucleotides, but becomes problematic when incorporating C8-aryl-dG adducts into longer strands that are suitable substrates for primer extension assays. Thus, we developed a solid-phase DNA synthesis approach that uses modified phosphoramidites for the generation of modified DNA substrates. This is now our method of choice for incorporation of C8-aryl-dG adducts into oligonucleotides.

Who are the main contributors to this project? How are their expertise and the University of Guelph assisting you in your endeavours?

The main contributors to my research are graduate students (PhD and MSc students) and fourth-year undergraduate (BSc) students from the Departments of Chemistry and Toxicology at the University of Guelph. Currently, Professor Mei Li is a visiting scholar from Nanjing University in China and she is also contributing to our research. The University of Guelph provides teaching assignments for my graduate students that helps to pay their salaries and provides major instrumentation, including Nuclear Magnetic Resonance and Mass Spectrometry facilities, that we use on a regular basis to characterise nucleoside adducts and oligonucleotides modified with the C8-aryl-dG adduct.

Professor Richard Manderville discusses his work on 2’-deoxyguanosine, an organic compound which could be used in biosensors due to its fluorescence, with numerous applications for studying DNA.
Disregarded dG

Despite its potential in industry, from biosensing products to cancer treatment, 2'-deoxyguanosine (dG) has been largely overlooked. With a renewed vigour, researchers at Canada’s University of Guelph have begun to unravel the possibilities presented by this exciting compound.

2'-DEOXYGUANOSINE (dG) is a purine 2'-deoxyribonucleoside with a guanine nucleobase, and has been an important tool in the study of oxidative stress and cancer research. When dG is modified at its eight-carbon atom by aryl residues, it forms C8-dG adducts, an organic compound which offers a wide range of applications, from healthcare to agriculture. The abilities of C8-dG adducts to bind with oligonucleotides, molecules which bind sequentially to DNA and coordinate with various transition metals, is a major benefit to researchers, since these adducts are fluorescent and can be used as complex biomarkers.

With these key applications, C8-dG adducts within oligonucleotides could be utilised as restriction enzymes, cutting longer pieces of DNA at target points in order to provide novel therapeutics. Similarly, such DNAzymes could act as chiral catalysts in aqueous solutions, and the compounds used to form metallo-DNAs, creating nanomaterials with unique properties.

Yet in many studies, C8-dG adducts have been overlooked in favour of other compounds. The molecules are similar to nitrogen-linked adducts – formed by arylamine and related substances – which are well-established human carcinogens. Consequently, these molecules have received the majority of attention within toxicological research.

Due to the direction of funding and research, the potential benefits of C8-aryl-dG adducts have previously been neglected by investigators, leading to a lack of information on the full potential of these compounds.

A NEW APPROACH

This bias is being challenged by a research group at the University of Guelph in Canada, led by Professor Richard Manderville. Starting in 2005, they began to change the predominant trend by investigating C8-aryl-Purine DNA adducts. Adopting a highly collaborative approach, and with funding from the National Sciences and Engineering Research Council (NSERC), the team has already uncovered a range of wider uses for the molecule.

DAZZLING CHEMISTRY

The work is currently being closely shared between the researchers at the University of Guelph, and their partners at Switzerland’s Institute of Food, Nutrition and Health within ETH. The process begins with the generation of various DNA substrates that contain C8-aryl-dG adducts. These are then sent to the Swiss laboratory, which is run by Professor Shana Sturla. Here, the investigators are able to map the DNA, determining how the adducts are processed by polymerase enzymes that are instrumental in ‘reading’ DNA.

Concurrently, the group at the University of Guelph will use the fluorescence of C8-aryl-dG adduct to uncover how DNA polymerase binds to the molecule. Manderville is leading the Canadian group, and explains how C8-aryl-dG is playing an important role in improving understanding in this area: "Most DNA adducts lack fluorescence, but this unique property of C8-aryl-dG adducts can be utilised to provide a deeper understanding of how these lesions influence DNA polymerase function”.

The fluorescence, which is central to the prospects of adducts is also part of the team’s current investigations into their biosensing capabilities. The researchers have been incorporating C8-aryl-dG adducts into aptamers, molecules which bind to specific target molecules, such as food toxins and proteins that are potential biomarkers of human diseases. Similarly, the fluorescence is anticipated to be useful in the creation of modified oligonucleotides, polymers which bind in site-specific ways to DNA and RNA. While this is still to be fully investigated, there is hope that it will prove another useful function of C8-aryl-dG adducts.

COMBATTING CANCER

Another avenue being pursued by the investigators at the University of Guelph has...
INTELLIGENCE

CHEMICAL AND BIOCHEMICAL PROPERTIES OF C8-ARYL-PURINE DNA ADDUCTS

OBJECTIVES

To fill the gap in our understanding of the consequences of C8-Aryl-purine adduct formation by characterising their impact on duplex DNA structure and replication, and to assess their chemical creativity.

KEY COLLABORATORS

Professor Shana Sturla, Institute of Food, Nutrition and Health, Switzerland
Dr Wojciech Gabryelski, University of Guelph, Canada
Dr Annie Pfohl-Leszkowicz, National Polytechnic Institute of Toulouse, France
Dr Stacey Wetmore, University of Lethbridge, Canada

FUNDING

Natural Sciences and Engineering Research Council of Canada (NSERC)

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RICHARD A MANDEVILLE received his BSc degree in chemistry and biology from Queen’s University in Kingston, Canada and his PhD in physical organic chemistry from Queen’s University under the supervision of Professor Erwin Buncel. After postdoctoral training with Professor Sidney M Hecht at the University of Virginia, in 1995 he joined the faculty in the Department of Chemistry at Wake Forest University in Winston-Salem, North Carolina, serving as Assistant and Associate Professor. On 1 July 2004, he returned to Canada and joined the Department of Chemistry at the University of Guelph in Ontario, serving as Associate, full Professor and Director of the Toxicology Program. His current research interests include DNA adduction by phenolic toxins and biosensing applications of modified fluorescent DNA bases.

been laying the groundwork for the use of C8-aryl-dG adducts as novel anticancer drugs. Once again, it is the fluorescence of the adduct that is central to this mechanism. The researchers have been able to generate new derivatives of the adducts which possess quenched fluorescence in water, but can be selectively excited within DNA. When the C8-aryl-dG adduct is folded into the DNA structures the fluorescence switches on. This in turn works as a sensor, which goes off when a molecular target is bound to by the oligonucleotide. These targets can include certain proteins, or environmental toxins, meaning that the modified oligonucleotides have the potential to act as sensors for particular molecules which are cancer risks.

Certain C8-aryl-dG adducts also undergo selective oxidation in the presence of singlet oxygen. Singlet oxygen is an electronically-excited state of molecular oxygen and is used to treat cancer through photodynamic therapy. Modified C8-aryl-dG adducts could become powerful tools in combination therapies for cancer treatment. Singlet oxygen specifically allows the C8-aryl-dG adducts to be oxidised photochemically. This results in the creation of an oxidised base, which then reacts with other proximate DNA bases in order to form DNA cross-links. These are essential for the potential functionality against cancer, as Manderville elaborates: “Many anti-cancer drugs kill cancer cells though DNA cross-link formation, so it is expected that certain C8-aryl-dG adducts will facilitate cell death once oxidised by singlet-oxygen”. Fitting into a class of existing anticancer drugs, the potential for C8-aryl-dG adducts as therapeutics is an important part of the work the team is completing.

BOUND TOGETHER

Collaboration has been of central importance throughout the seven years that the project has been running. Within the department at Guelph, Manderville has been closely working with Dr Wojciech Gabryelski. Further afield, he has been completing investigations with Dr Annie Pfohl-Leszkowicz of the National Polytechnic Institute of Toulouse (NPIT) on projects, which include being editors for the special issue of Toxins and jointly publishing three research articles. Moreover, this partnership has now led to student exchanges between the University of Guelph and NPIT. Finally, Dr Stacey Wetmore, from the University of Lethbridge in Alberta, has been instrumental in increasing the understanding of C8-aryl-dG adduct formation within DNA. Using cutting-edge computer models, Wetmore’s laboratory seeks to understand the structures and reactivity of C8-aryl-dG adducts, and has led to the publication of seven joint articles.

Alongside the investigative work, the NSERC support has led to the training of eight graduate and 25 undergraduate students, a process which is still ongoing within the University of Guelph. With their funding currently up for renewal, the group is hopeful that a further endorsement from the funding body will allow their investigations to develop and that their work uncovering the role of C8-aryl-dG adducts will continue as strongly as it has begun.

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