INSIGHT INTO THE LOCATION OF DNA XENOBIOTIC DAMAGE BY MASS SPECTROMETRY

by

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A Senior Honors Project Presented to the

Honors College

East Carolina University

In Partial Fulfillment of the

Requirements for

Graduation with Honors

by

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May 2014

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ABSTRACT

Damage to DNA by a bioactivated xenobiotic typically occurs at specific sites within the genome, called hotspots. An example of this is benzo[*a*]-pyrene (BP), a xenobiotic that enters the body via cigarette smoking. Hotspots at which the final BP metabolite, (+/-)-*anti*-benzo[*a*]-pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), adducts DNA oligomers have been electrochemically detected. Determination of the number of adducts formed on the DNA oligomer from exposure to the xenobiotic is necessary for further insight into the damage reaction. Analysis of the damaged DNA samples by Electrospray Ionization Mass Spectrometry (ESI-MS) allows for observation of structural features by separation of the ions present by mass. Using tandem mass spectrometry to obtain data, MS/MS spectra are interpreted for the damaged DNA and used to determine the location of the damage by observing a corresponding change in mass due to the reaction of DNA with the xenobiotic. Tandem mass spectrometry allows for the isolation of certain peaks in the spectrum to further separate and compare to similar spectra of undamaged DNA. Further collection of spectra is necessary before preliminary findings can be reported.

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INTRODUCTION

Genotoxicity

Organisms are constantly exposed to foreign chemical substances, or xenobiotics, by means of pharmaceutical drugs, pollutants, or other natural compounds. As the body metabolizes these molecules in an effort to eliminate them, bioactivation occurs to introduce reactivity (Friedberg 2003). DNA and proteins are particularly vulnerable to damage as they can react with the activated xenobiotic species (Friedberg 2003). Genotoxicity refers to DNA damage by a reactive xenobiotic which can result in permanent mutations because persistent DNA adducts are not correctly repaired, causing errors upon replication (Feng *et al.* 2006). Rather than random DNA damage, genotoxicity by a bioactivated xenobiotic typically occurs at specific sites within the genome, called hotspots (Vousden & Lane 2007). Determination of genetic damage sites gives detailed information on disease origination.

Electrochemical Detection

Elucidation and detection of genomic hotspots have previously relied on the lengthy and complicated process of exposure of DNA or cells to the xenobiotic of interest, followed by DNA separation, enzymatic digestion, specialized polymerase chain reaction (PCR) amplification protocols, gel separation, and sequencing (Pfeifer & Riggs 1996). Faster and cheaper analysis methods have been developed to detect general genotoxicity reactions, such as oxidative damage or xenobiotic adduction (Palecek *et al*. 1998). Further exploration of new strategies is necessary to develop a high throughput platform to couple with DNA sequence genotoxicity detection since detailed disease etiological information can be determined from identified genetic damage locations (Satterwhite *et al.* 2011). Electrochemical hybridization sensors have excelled as one

such method to analyze xenobiotic related genotoxicity. By exploiting nanoscale interactions between the target in solution and a solid electrode surface, electrochemistry-based sensors offer sensitivity and selectivity for the detection of specific mutated genes associated with human disease (Drummond, Hill, & Barton 2003).

C12-viologen voltammetry is a function of the changes in DNA helical structure, indicated by the dramatic change in electrochemical signal in the presence of mismatched hybrids or single strand DNA (ssDNA) (Satterwhite *et al.* 2013). Recognition of two complementary strands allows for detection of short DNA sequences using various electrochemical transduction methods (Hvastkovs & Buttry 2009). The simplest approach involves the intercalation of a groove-binding molecule to provide a characteristic signal when double stranded DNA (dsDNA) is present. This allows for the electrochemical detection by monitoring the redox-active molecule as it interacts with DNA (Satterwhite *et al.* 2011). Even though more sensitive methods exist, this method yields valuable information on DNA structure since the electrochemical signals are directly affected by mismatches and protein binding interactions that alter π-stacking (Satterwhite *et al.* 2011). To support the electrochemical sensor findings and provide further insight into the damage reaction other instrumental methods, such as mass spectrometry, are used to determine the number of adducts formed on the DNA oligomer from exposure to the xenobiotic.

Tandem Mass Spectrometry

Electrospray Ionization Mass Spectrometry (ESI-MS) is an important and powerful instrumental tool for the analysis of biomolecules. Structural information regarding the primary sequence of a nucleic acid, such as a protein or DNA, via ESI-MS relies primarily on the

dissociation behavior of the ions formed by electrospray ionization (Wu & McLuckey 2004). This ionization technique is especially useful in producing ions from macromolecules because it overcomes the propensity of these molecules to fragment when ionized. Additionally, ESI is advantageous over other atmospheric pressure ionization processes since it can produce multiply charged ions, which extends the mass range of the analyzer to accommodate the large order of magnitude values observed in proteins and their associated polypeptide fragments (Glish & Vachet 2003). Gas phase analysis of biological molecules in solution is possible using this instrumental analysis method because important noncovalent interactions are preserved (Wilson & Brodbelt 2007).

Tandem mass spectrometry (MS/MS) involves two stages of ion separation by mass spectrometry. The first stage involves the isolation of ions of a desired m/z value from the rest of the ions that enter the instrument from the ion source. These isolated ions are called parent ions and are subjected to a chemical reaction that causes a change in either the mass or charge value (Glish & Vachet 2003). Collision-induced dissociation (CID) is one such mechanism used to fragment molecular ions in the gas phase by acceleration through an electrical potential to high kinetic energy. Collision of the accelerated ions with neutral molecules, such as helium gas, converts some of the kinetic energy into internal energy. Bond breakage occurs dissociating the molecular ion into smaller fragments. The fragmented ions created are called product ions and are analyzed with the second stage of MS/MS (Glish & Vachet 2003).

MS/MS experiments are used to study DNA oligomers exposed to xenobiotics to determine the number of adducts formed and gain insight into the damage reaction (Satterwhite *et al.* 2011). Fragmentation or dissociation patterns for oligonucleotides show large variations dependent upon their charge state distributions (Pomerantz, Kowalak, & McCloskey 1993).

Comparison of the charge state distribution patterns of xenobiotic-damaged and undamaged DNA in the resulting spectra offers insight into the damage reaction (Glick *et al.* 2009). ESI-MS is used in validation of electrochemical detection methods to further probe the adduct structure of genotoxicity and support information determined electrochemically (Satterwhite *et al.* 2011).

Benzo[a]pyrene

Benzo[a]pyrene (BP) is a well-studied xenobiotic that enters the body via cigarette smoking and damages DNA at specific sites within the genome. Bioactivation of this compound within the body as it is metabolized results in the reactive stereoisomer metabolic products, (+/-)-anti-benzo[a]-pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) (Figure 1). Hotspots at which the final BP metabolite adducts DNA oligomers have been electrochemically detected within the *TP53* gene at site-specific locations. The p53 protein, which is involved in cellular apoptosis, is coded for by the *TP53* gene (Vousden & Lane 2007). Specifically BPDE adducts guanines at codons 157, 248, and 273, affecting transcription by altering the amino acids forming the DNA binding region of the p53 protein (Satterwhite *et al.* 2011). DNA mutations at the particular codons commonly adducted by BPDE are prevalent in many cancers (Vousden & Lane 2007).

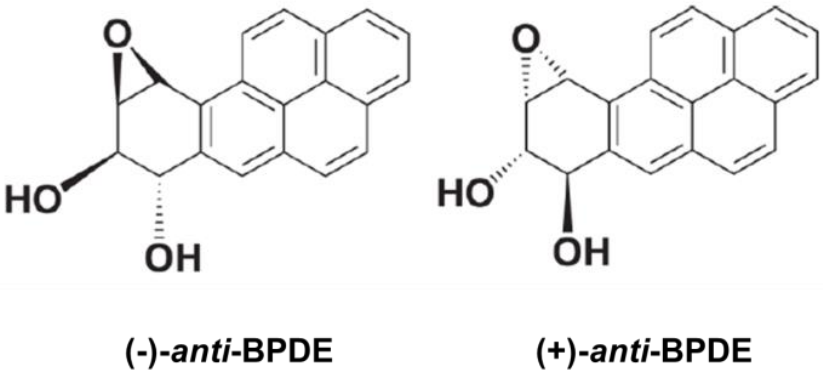

Figure 1: Chemical structures of *anti*-BPDE stereoisomers.

Project Goal

The main analytical goal was to detect carcinogenic genotoxicity caused by BPDE at a specific gene sequence. This biophysical – analytical method consisting of electrochemical sensor studies followed by further mass spectrometry experiments allows for insight into an important genotoxic process of a well-known carcinogen, BPDE. Even though more sensitive methods for complementary target detection exist, this instrumental analysis method allows for the collection of valuable DNA oligomer structural information. A set of spectra for BPDE damaged DNA and a set for undamaged DNA are both necessary. Comparison of these spectra allows for the determination of the oligonucleotide fragmentation patterns based on the charge state distributions. Important information on the number of DNA adducts formed during the damage reaction could have been determined. The structural information obtained through the mass spectrometric studies would have then been combined with the electrochemical trends to provide insight into the damage location of BPDE.

MATERIALS & METHODS

Materials

DNA oligomer sequences used were wt. 273, 5'-TTT GAG GTG CGT GTT TGT GCC-3', and wt. 273 complement, 5'-GGC ACA AAC ACG CAC CTC AAA-3'. Samples were diluted in 50:50 methanol:water before ESI-MS analysis.

Electrospray Ionization Mass Spectrometry

All samples were analyzed by nanoESI-MS using a custom-built source on a Bruker Esquire 3000plus quadrupole ion trap mass spectrometer (Bruker Daltonics, Billerica, MA) in negative mode ESI. The potential difference between the emitter and source aperture was optimized around 1 kV to avoid sample arcing at higher potential difference values. Heated nitrogen gas (250 °C) was used to aid in nebulization and desolvation. The flow of nebulizer gas was controlled to minimize fragmentation during ionization and vaporization

RESULTS

Analysis of DNA samples by ESI-MS offered insight into the damage reaction by determination of the number of adducts formed on DNA oligomers via xenobiotic exposure. Comparison of the charge state distributions in mass spectra of BPDE damaged DNA to those of undamaged DNA offers insight into damage reaction by observation of a corresponding change in mass due to the reaction of DNA with the xenobiotic (Figure 2).

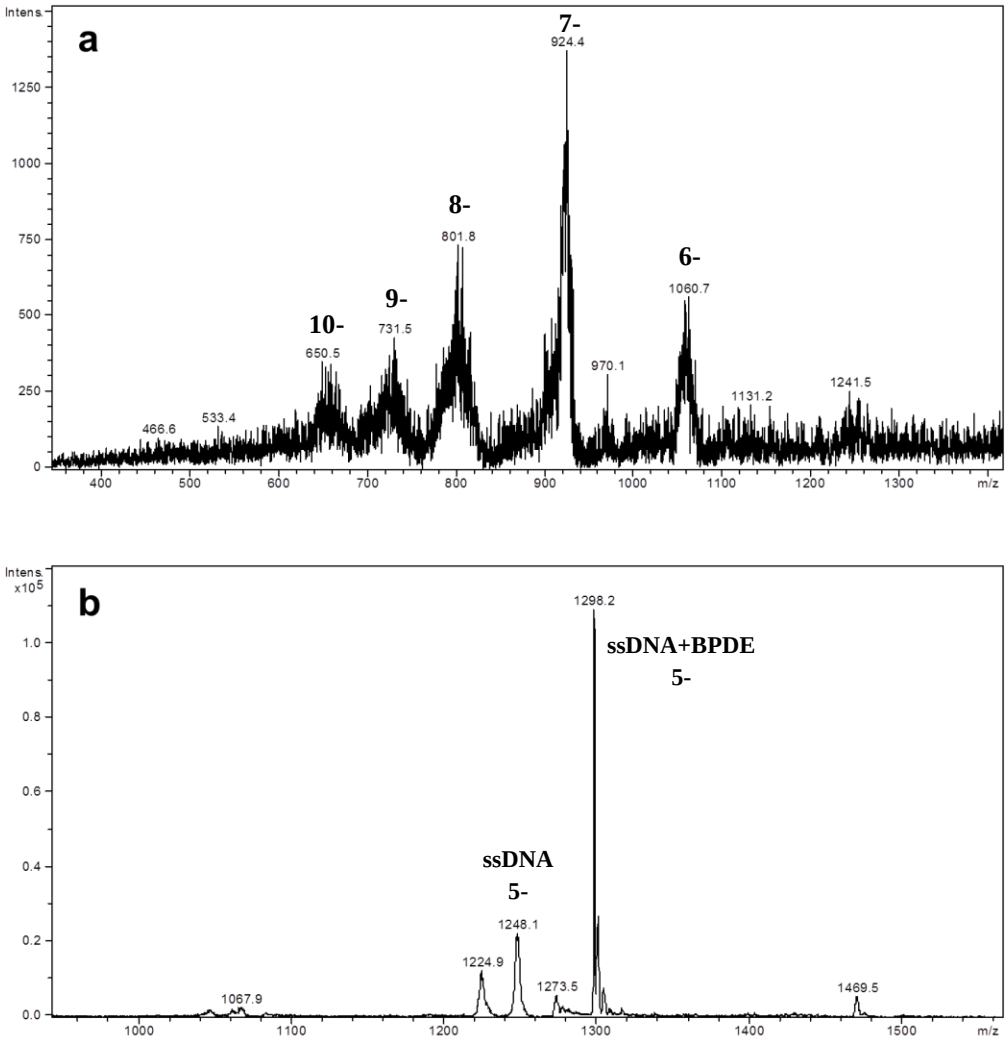

Figure 3: Sample mass spectra of (a) undamaged and (b) BPDE damaged DNA with labeled charge state distributions.

It was of interest to determine the number of adducts formed on DNA oligomers via xenobiotic exposure in order to gain insight into the damage reaction. Figure 2 represents mass spectra of the charge states, with the charge state distributions labeled, found in (a) undamaged DNA with wt. 273 and (b) a typical BPDE reaction with wt.273. Optimization of the solvent and MS conditions used promoted more efficient desolvation and dissociation of the sample into two ssDNA. This allowed for larger signals and a clearer distribution in the collected mass spectra.

Nevertheless, arcing during sample introduction, solvent cluster chemical noise, and low ion transmission all represent challenges faced when optimizing conditions and collecting spectra. Electrical discharge from the capillary tip, or corona discharge, results in the chemical ionization of the gas phase analyte and solvent molecules that are present in the space around the ESI spray capillary (Cech & Enke 2001). A mass spectrum obtained during corona discharge is often characterized by significant amounts of background noise, due to ionized solvent molecules, and poor stability (Cech & Enke 2001). In the corona discharge mode, arcing frequently occurs causing a loss in ESI current. Electron-scavenging gases or halogenated solvents suppress corona discharge (Cech & Enke 2001). Protonated, sodiated, or ammoniated solvent clusters are common chemical noises observed in ESI mass spectra and are a particularly prevalent source of interference at low masses (Cech & Enke 2001). When the solvent is more volatile than the ESI analyte, it is usually possible to reduce the mass spectral response of solvent clusters by heating the electrospray droplets before they enter into the high vacuum region of the mass spectrometer. Sensitivity in ESI-MS is greatly affected by ion transmission as a function of the efficiency by which molecules are converted into gas-phase ions and the efficiency by which these gas-phase ions are transferred through the various stages of the mass spectrometer and detected (Cech & Enke 2001). These challenges make the optimization of sample introduction

and mass spectrometry instrument parameters necessary for the analysis of xeniobiotic-damaged biomolecules.

CONCLUSION

Electrochemical genotoxicity detection offers a cost effective and high throughput method for detection of carcinogenic BPDE-related DNA damage at a specific gene sequence. Supporting studies carried out using this instrumental technique with tandem mass spectrometry allows for further probing of the adduct structure and insight into the damage reaction. Collection of mass spectra of both BPDE damaged and undamaged DNA samples is necessary. Comparison of oligonucleotide fragmentation patterns based on the resulting charge state distributions offers insight into the hotspot locations of the genotoxicity. Combination of electrochemical detection and mass spectrometric analysis offers the most information about the damage reaction of a given xenobiotic.

The focus of this project was the optimization of the solvent and MS conditions in order to promote more efficient desolvation and dissociation of the sample, allowing for larger signals and a clearer charge state distribution in the experimentally collected mass spectra. Arcing during sample introduction, solvent chemical noise, and low ion transmission represented the largest challenges faced when optimizing conditions and collecting spectra. Further collection of mass spectra of BPDE damaged DNA is necessary for comparison to the spectra of undamaged samples. Combination of the DNA adduct structure information with the electrochemical detection results will provide insight into the hotspot location of the DNA damage and valuable etiological information on the carcinogenic xenobiotic, BPDE.

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