High-throughput Measurement of DNA Mono- and Oligo-nucleotides by RapidFire/TOF-MS

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Introduction

Measuring structural changes to DNA is pivotal to understanding epigenetics, gene expression, and cancer. Accordingly, instrumentation that facilitates the analysis of DNA reactants is valuable to discovering drugs with wide therapeutic potential. This work contains three demonstrations in which the Agilent RapidFire High-throughput Mass Spectrometry System was used to measure mono- and oligo-nucleotides from *in vitro* samples, establishing the viability of the platform for DNA-based applications. The RapidFire system performed sample preparation, from 96- and 384-well plates, with a sustained throughput of ~8 seconds per sample, and permitted the DNA species to be measured directly by online time-of-flight (TOF) mass spectrometry (MS). TOF-MS collects full scan data over time and thereby permitted the abundance of any given DNA species to be extracted from the data set post run.

In the first demonstration, a set of four mononucleotides was analyzed by RapidFire/TOF-MS, highlighting the potential for investigations including detection, quantitation, and localization of modified bases in DNA of known sequence. In the second demonstration, the AlkB enzyme was used to repair an etheno adduct, located within a 16-mer oligonucleotide, and the reaction products (including substrate, two repair intermediates, and product DNA strands) to be measured discretely. Lastly, in the third demonstration, the abundance of a single 5-methylcytosine residue, located within a 20-mer oligonucleotide, was measured in the presence of native 20-mer DNA. Taken together, this research provides a framework for high-throughput detection of DNA species by MS, in general, and can be used to accelerate experimentation in multiple fields.

1. Measuring Mono-nucleotides

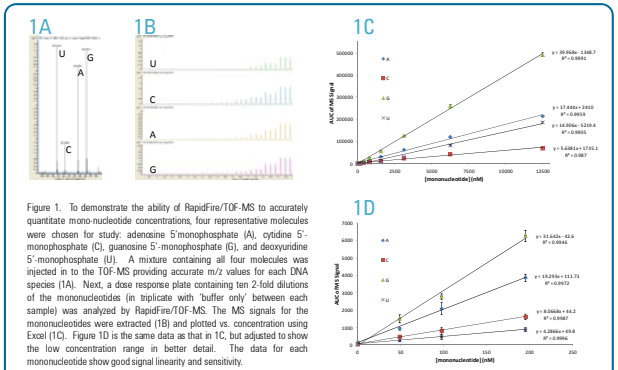


Figure 1. To demonstrate the ability of RapidFire/TOF-MS to accurately quantitate mono-nucleotide concentrations, four representative molecules were chosen for study: adenosine 5'monophosphate (A), cytidine 5'-monophosphate (C), guanosine 5'-monophosphate (G), and deoxyuridine 5'-monophosphate (U). A mixture containing all four molecules was injected in to the TOF-MS providing accurate m/z values for each DNA species (1A). Next, a dose response plate containing ten 2-fold dilutions of the mononucleotides (in triplicate with 'buffer only' between each sample) was analyzed by RapidFire/TOF-MS. The MS signals for the mononucleotides were extracted (1B) and plotted vs. concentration using Excel (1C). Figure 1D is the same data as that in 1C, but adjusted to show the low concentration range in better detail. The data for each mononucleotide show good signal linearity and sensitivity.

2. Measuring Oligo-nucleotides (Example 1)

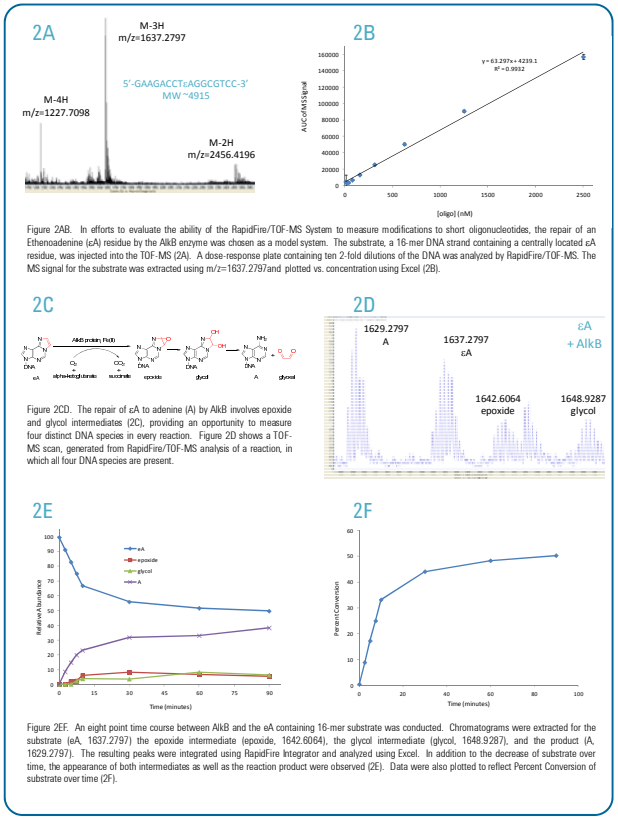


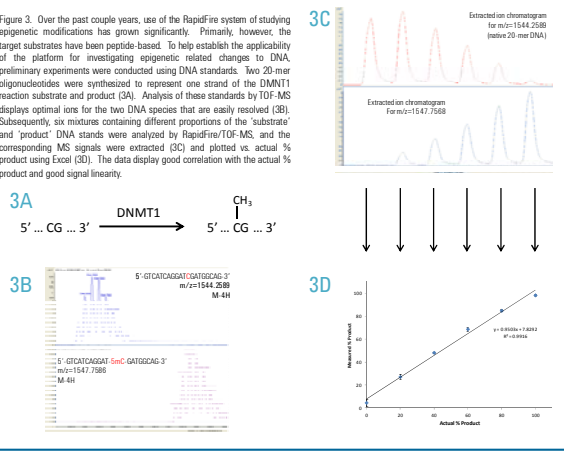
Figure 2AB. In efforts to evaluate the ability of the RapidFire/TOF-MS System to measure modifications to short oligonucleotides, the repair of an Ethenoadenine (εA) residue by the AlkB enzyme was chosen as a model system. The substrate, a 16-mer DNA strand containing a centrally located εA residue, was injected into the TOF-MS (2A). A dose-response plate containing ten 2-fold dilutions of the DNA was analyzed by RapidFire/TOF-MS. The MS signal for the substrate was extracted using m/z=1637.2797and plotted vs. concentration using Excel (2B).

Figure 2CD. The repair of εA to adenine (A) by AlkB involves epoxide and glycol intermediates (2C), providing an opportunity to measure four distinct DNA species in every reaction. Figure 2D shows a TOF-MS scan, generated from RapidFire/TOF-MS analysis of a reaction, in which all four DNA species are present.

Figure 2EF. An eight point time course between AlkB and the εA containing 16-mer substrate was conducted. Chromatograms were extracted for the substrate (εA, 1637.2797) the epoxide intermediate (epoxide, 1642.6064), the glycol intermediate (glycol, 1648.9287), and the product (A, 1629.2797). The resulting peaks were integrated using RapidFire Integrator and analyzed using Excel. In addition to the decrease of substrate over time, the appearance of both intermediates as well as the reaction product were observed (2E). Data were also plotted to reflect Percent Conversion of substrate over time (2F).

3. Measuring Oligo-nucleotides (Example 2)

Figure 3. Over the past couple years, use of the RapidFire system of studying epigenetic modifications has grown significantly. Primarily, however, the target substrates have been peptide-based. To help establish the applicability of the platform for investigating epigenetic related changes to DNA, preliminary experiments were conducted using DNA standards. Two 20-mer oligonucleotides were synthesized to represent one strand of the DMNT1 reaction substrate and product (3A). Analysis of these standards by TOF-MS displays optimal ions for the two DNA species that are easily resolved (3B). Subsequently, six mixtures containing different proportions of the 'substrate' and 'product' DNA stands were analyzed by RapidFire/TOF-MS, and the corresponding MS signals were extracted (3C) and plotted vs. actual % product using Excel (3D). The data display good correlation with the actual % product and good signal linearity.



Conclusions

1. The RapidFire/TOF-MS system enables the measurement of mono- and oligo-nucleotides from *in vitro* mixtures at a sustained rate of ~8 seconds per sample.
2. Experiments with mono-nucleotides demonstrate efficient resolution of the different DNA species, good sensitivity, low carryover, and a linear signal response with dose.
3. Experiments with oligo-nucleotides demonstrate the ability to monitor enzymatic transformation of multiple DNA species concurrently, facilitating the identification and quantitation of all reaction products.
4. Because high-throughput detection of DNA species by MS is fast, accurate, and versatile, it has the potential to accelerate a broad range of drug discovery efforts.

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