WP 396

Capillary Electrophoresis Mass Spectrometry (CEMS) of Oligonucleotides using a 908Devices ZipChip

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Introduction

- Ion Pair Reverse Phase (IPRP) chromatography is the current gold standard for characterization of oligonucleotides and their impurities and degradants. However, it is always desirable to have an orthogonal method for additional and complementary data to standard IPRP chromatography. In this presentation we explore the use of a 908Devices ZipChip coupled to a high resolution mass spectrometer as an orthogonal method to chromatography for oligonucleotide analysis.

- The highest resolution chromatographic separations typically require ion pair combinations such as triethylamine (TEA) and hexafluoroisopropanol (HFIP) with negative ion detection by mass spectrometry. The 908Devices ZipChip can ionize oligonucleotide analytes without the need for reagents such as HFIP and TEA. Here we present data obtained from a Thermo LUMOS high resolution mass spectrometer using a 908Devices ZipChip interface.

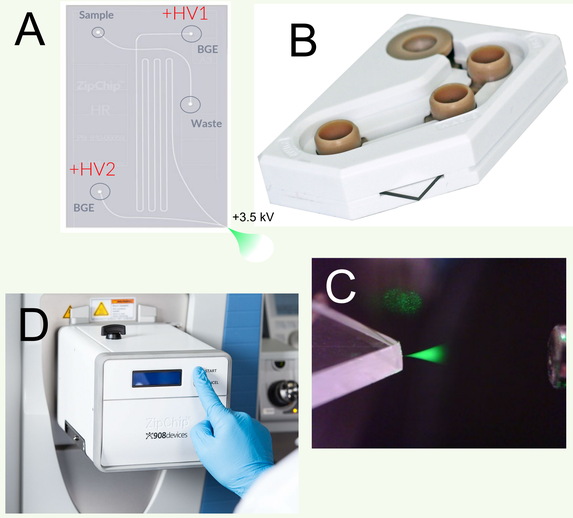

Figure 1. Clockwise from the Top: The 908Devices ZipChip schematic (A) with a photo of a ZipChip cartridge (B) shown with an electrospray plume (C) from the glass emitter and the ZipChip interface mounted on a mass spectrometer (D).

Methods

- Samples:
  - Agilent Oligonucleotide Resolution Standard (Part No. 5190-9028)
  - Fully modified (Dicerna) siRNA oligonucleotide

All oligonucleotides were dissolved in LCMS grade water at various concentrations (approximately 0.5 – 0.05 mg/mL for the siRNA and 400 fmol/uL to 8 pmol/uL for the Agilent RNA resolution standard).

- ZipChip: The higher resolution HRB chip was used with a proprietary background electrolyte (BGE) solution designed for analysis of oligonucleotides. Sample injection volume was either 1 or 10 nL. Field Strength was 500 V/cm.

- Mass Spectrometer: Thermo Lumos used in either MS or MSMS mode at mass resolutions ranging from 30 to 240K. Mass range was 1000-4000 for MS and 300-4000 for MSMS. MSMS was done using either Collision Induced Dissociation (CID) or higher-energy collisional dissociation (HCD). The ZipChip ionizes oligonucleotides as positive ions.

CE-MS of siRNA Duplex

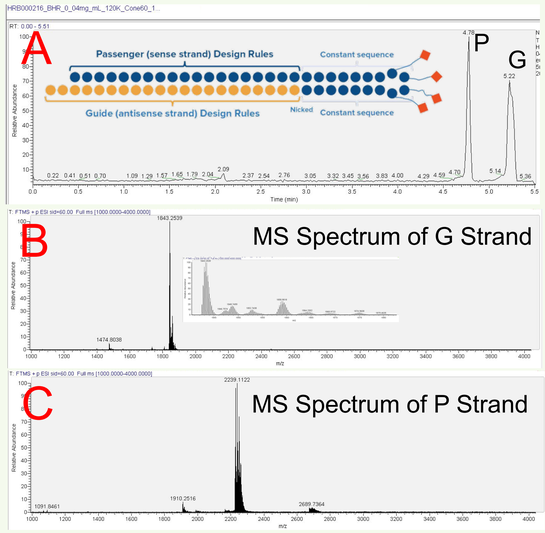

Figure 2. A. Total Ion Electropherogram (TIE) from a 1 nL injection of 0.04 mg/mL Dircerna GalXC siRNA duplex. The duplex dissociates into the shorter guide (G) strand shown in orange and the longer passenger (P) strand shown in dark blue. The strands are well separated within 6 minutes with the longer P strand having a shorter migration time. B. MS spectrum of G with a zoomed view of the protonated species and adducts. C. MS spectrum for P.

The CE-MS total ion electropherogram of an siRNA duplex (1 nL injection of a 0.04 mg/mL solution) is shown in Figure 2A. Our siRNA duplex molecule denatured under the Oligonucleotide BGE conditions which resulted in two peaks being detected for each strand (no duplex was observed). The mass spectra for G and the longer P single strands are shown in Figures 2B and 2C, respectively. Both strands ionize with a single predominant charge state observed (+4 for G and +6 for P). Besides the protonated species alkali metal adducts are also observed with the adducts more abundant with the longer P strand. This is most likely due to the higher number of phosphate groups on the longer P strand.

Sequence verification of these synthetic modified strands is important and typically accomplished by acquiring MSMS spectra. The modifications make the strands more resistant to fragmentation which typically requires many MSMS spectra of different charge states and different collision energies to be acquired to achieve full sequence coverage.

CE-MSMS of siRNA G Strand

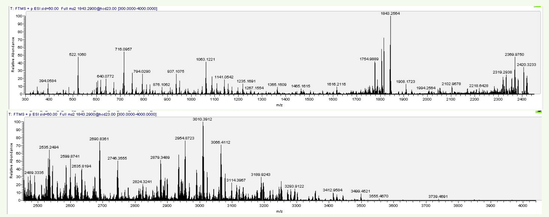

Figure 3. HCD fragmentation of shorter G strand showing information rich MSMS spectrum of + 4 charge state *m/z* 1843 (acquisition was at 240K resolution).

- The MSMS spectrum from the shorter G strand (Figure 3) contains abundant fragment ions with charge states ranging from +1 to +3. HCD fragmentation of the predominant charge state ion for the G strand yielded complex and information rich MSMS spectra.

- The high resolving power (MSMS was acquired at 240K mass resolution) of the Lumos mass spectrometer facilitates deconvolution and interpretation of the fragment ions to allow nucleotide sequence verification from a single MSMS spectrum.

- The HCD mass spectrum (Figure 4) of the longer P strand (~ 13.5 kDa) still contains abundant ions but P is more difficult to fragment compared to the shorter G strand resulting in fewer informative fragment ions above *m/z* 2000.

CE-MSMS of siRNA P Strand

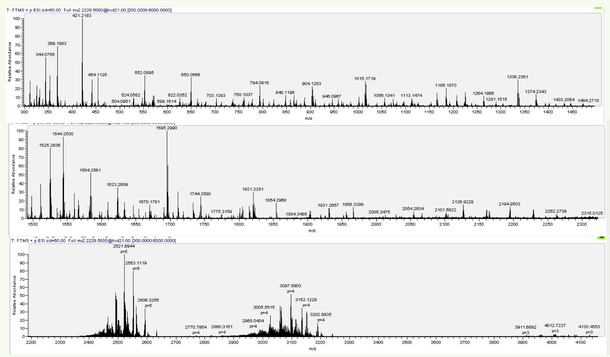

Figure 4. HCD fragmentation of the 6+ charge state of the longer P strand. While the MSMS spectrum below *m/z* 2000 is still complex and information rich, the ions above *m/z* 2000 are less informative with isolated charge state “islands”.

CE-MSMS of RNA (Agilent Standard)

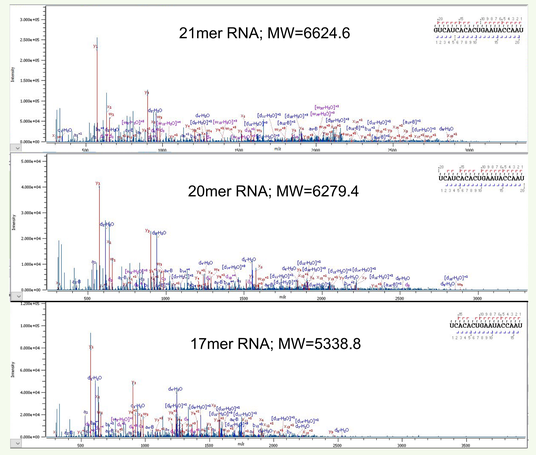

Figure 5. HCD MSMS spectra of the three longest (unmodified) RNA strands in the Agilent oligonucleotide resolution standard. The fragment ion assignment / annotation was done using the Oligo module of Protein Metrics (Cupertino, CA) software with sequence coverage shown graphically for each strand. The abundant ions not annotated are assumed to be due to base loss.

Summary / Conclusions

- CE-MS of oligonucleotides (with ZipChip)
  - Positive ions easily generated even for longer strands (e.g., >10 kDa)
  - Rapid analysis time (~ 6 minutes per run)
  - Most of the signal is confined to a single charge state (1-3 charge states observed)
  - Highly sensitive analysis (optimal concentrations of ~ 0.05 mg/mL or ~ 400 fmol/uL) with only 1 nL injection volume
  - No need for IPRP reagents (e.g. HFIP, TEA)

- CE-MSMS with Thermo Lumos HRMS
  - HCD provides information rich MSMS spectra with high complexity requiring a mass spectrometer with high resolving power for deconvolution/interpretation.
  - Positive ion MSMS fragmentation appears to be similar to negative ion but with more base losses observed
  - Not necessary to do MSMS of many different charge states due to one predominant multiply charged species for sequence verification

- Work currently in progress
  - Verification of unannotated fragments
  - Refinement of BGE to improve resolution
  - Reduction of alkali metal adducts