Comprehensive Characterization of tRNA by Intact Mass Analysis

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INTRODUCTION
Transfer ribonucleic acid (tRNA) from the DNA gene by the multienzyme complex RNA polymerase III (pol III) and processes through a series of maturation steps resulting in a tRNA having an average mass of 25kDa and ranging in length of ~70-100 nucleotides. Of all classes of RNA, the tRNA contain the greatest density of post-transcriptional modifications ranging from simple methylation to hyper modifications having multi-enzymatic biophysical pathways. Structurally, tRNA contain four nucleotide bases pairs, the codon of the mRNA during translation, the D-Loop, the TCV loop and the acceptor stem which contains the 3' sequencedCCA. The terminal TCA carries the amine specific to the RNA and is placed onto the tRNA by the RNA's cognate protein complex, the aminoacyl tRNA synthetase (aaRS).

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) of enzymatic digests has historically been the gold standard to studying tRNA's and their modifications. Improvements in instrumentation, as well as mass spectrometric sensitivity and mass accuracy, now enable routine analysis of RNA oligonucleotides ranges up to 200nm. Here, using UPLC-MS/MS we show the ability to fully characterize a tRNA through deconvolution of an intact mass spectral peak envelope. Using the commercial software Accucore™ from Sciex, we identified both intact nucleosides, a base substitution in the acceptor stem, and show they could not be truncated in a to a gCPp 3' terminal. We further are able to identify a possible stable intermediate in the Wycoffius biosynthetic pathway, a 7 terminal phosphorylated adenosine as well as identify possible intermediates in acceptor stem modification with monoiotic mass errors ±5ppm.

Confirmation of identification was through traditional mass spectrometric nucleoside and mass mapping experiments.

MATERIALS AND METHODS

Liquid Chromatography
Intact and oligonucleotide separations were accomplished by reversed-phase liquid chromatography using a Thermo Scientific™ UHPLC system. Nucleoside separations were accomplished by reverse-phase liquid chromatography using a Thermo Scientific™ Accucore™ C18, 1.5 µm, 2.1 x 100 mm column on a Thermo Scientific™ Vanquish™ Flex UPLC system using ammonium acetate buffers.

Elution gradient for intact mass spectra at 10% B from 0 to 0.5%, 40% B at 7.0 min, 30% B at 7.1 h for 0.5 min, returning to 10% B at 7.51 min. A flow rate of 400 µL/min. The column temperature was set at 80 °C.

Elution gradient for oligonucleotide mass mapping starts at 0% B from 0 to 1 min., 30% B at 10-18 min, 90% B at 16.1 min. hold for 1 min. then returning to 0% B at 17.1 min.

Elution gradient for nucleoside analysis starts at 0% B (from 0 to 3 min), 35% B at 10-13 min, 95% B at 14 min. hold for 1 min. then returning to 0% B at 15.1 min.

Mass Spectrometry
HRAM analyses were performed on an Thermo Scientific™ Orbitrap™ Accurate FIA mass spectrometer interfaced with a heated electrospray (HESI) source in negative polarity. Full scan data was acquired in Intact Protein mode at low pressure using a resolution of 240,000 at 400 m/e, mass range 405-3000 m/e, automatic gain control (AGC) target 1 x 10^6, and maximum injection time (IT) 120 ms. The other instrumental conditions were quadrupole isolation of ±1.2 m/z; radio frequency (RF) 35%; sheath gas, auxiliary gas, and sweep gas of 50, 10 and 1 arbitrary units, respectively; ion transfer tube temperature of 350 °C; vaporizer temperature of 300 °C; and spray voltage of 3500 V. Data was acquired using Thermo Scientific™ Xcalibur™ 4.5 and analyzed with Thermo Scientific™ Freeware™ 1.8 and ThermoScientific™ BioFinder™ 5.1.

RESULTS

Intact mass measurement of tRNA shows a charge distribution state of 9:1 to 35:1, with the most abundant charge ratios between 15:1 and 20:1 (Figure 1A). For each charge state several features are observed with a minimum and maximum relative abundance between 3:1 and 20:1. Deconvolution of the spectra from the mass chromatographic peak reveals several masses, the most abundant having a mass of m/z 495.0138 (Figure 1B). The baseline peak is split into two with the 17-charge state enhancing the ability of the software to deconvolute the spectra (Figure 1C).

One of the most abundant signals in the deconvolution results is identified as a demethylation of the A68 tRNA. Looking at the most probable table methylation suggests a loss of methylation on the Wycoffius (W) site, which would result in a monomeric mass of 494.1701 Da. To verify if the demethylated signal in the deconvolution result was the yW-14 intermediate, isotope ion fragmentation (IE) in the nucleotide data for the protonated mass were generated and returned a feature at ~10.6 ppm, a precision, and a yW (Figure 2A). Examining the collision induced dissociation (CID) and High Energy Collision Dissociation (HCD) data for the yW-14 (Figure 2B and 2C) yielded fragmentation consistent with a yW-14 structure.

CONCLUSIONS
In these experiments we have used ion pairing reverse phase liquid chromatography coupled to accurate mass spectrometry to identify an intact tRNA through deconvolution of its negative charge state distribution. We were further able to identify the known tisoeder as well as an under modified extent. We further show for the tisone, the tisoeder species exist as a single 2 truncated species CPsp with other low abundant identities characterized as being intermediates in tRNA maturation.

REFERENCES
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