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RNase mapping of intact nucleic acids by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICRMS) and ^{18}O labeling☆

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Abstract

We present a method using ^{18}O labeling and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICRMS) for characterizing intact ribonucleic acids (RNAs) and their posttranscriptional modifications. An ^{18}O label is incorporated at the 3′-phosphate end of oligonucleotides upon hydrolysis of RNAs with RNase T1. The ^{18}O-labeled digestion products are then identified without prior separation by ESI-FTICRMS. The combination of FTICRMS and ^{18}O labeling facilitate identification of RNase T1 digestion products and possible sites of posttranscriptional modification to RNAs. The mass measurement accuracy afforded by the use of FTICRMS, combined with the ^{18}O labeling of 3′-terminal phosphates and endonuclease specificity, extend the molecular weight range of oligoribonucleotides whose base compositions can be determined solely on the basis of mass measurement. High mass measurement accuracy also provides significant advantages for identifying the presence of posttranscriptional modifications, such as methylation. Additionally, RNase T1 digestion products can be isolated for further sequencing by sustained off resonance irradiation (SORI) collision-induced dissociation (CID). The presence of the ^{18}O label simplifies assignment of sequence specific oligonucleotide fragment ions and reduces the data reduction required for sequence interpretation. This approach can be used for RNA sequence verification and for RNA modification mapping.

Keywords: Isotope labeling; RNA sequencing; Posttranscriptional modifications; MS/MS; SORI; CID; tRNA; Oligoribonucleotide; Oligonucleotides; FTMS; FTICR; Methylations; RNase T1; Endonuclease

1. Introduction

The identification and characterization of posttranscriptional modifications are essential for fully understanding the functional roles of ribonucleic acids (RNAs). Mass spectrometry (MS) offers a number of advantages for the characterization of nucleic acids arising from its ability to provide mass and sequence information. As typically implemented, isolated RNAs are enzymatically digested with specific endonucleases prior to their analysis by MS or MS/MS approaches. The most popular approach has been the on-line separation and analysis of endonuclease digests using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) [1–12]. In addition, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been used wherein the endonuclease digest is analyzed without on-line separation [13–18].

Recently, Berhane and Limbach demonstrated that the incorporation of ^{18}O into RNase digestion products improves MALDI-based RNA mapping and posttranscriptional modification analysis of various RNAs [16]. As shown by the endonuclease cleavage mechanism (Fig. 1), an ^{18}O label can be incorporated into the final endonuclease digestion product if the enzymatic hydrolysis of RNAs takes place in the presence of ^{18}O-labeled water. Although MALDI-MS offers several advantages when analyzing complex mixtures of oligonucleotides arising from the generation of primarily singly charged ions and its higher tolerance towards salts typically present in oligonucleotide samples, the implementation of MALDI-based sequencing of oligonucleotides by post-source decay is usually more difficult than correspond-

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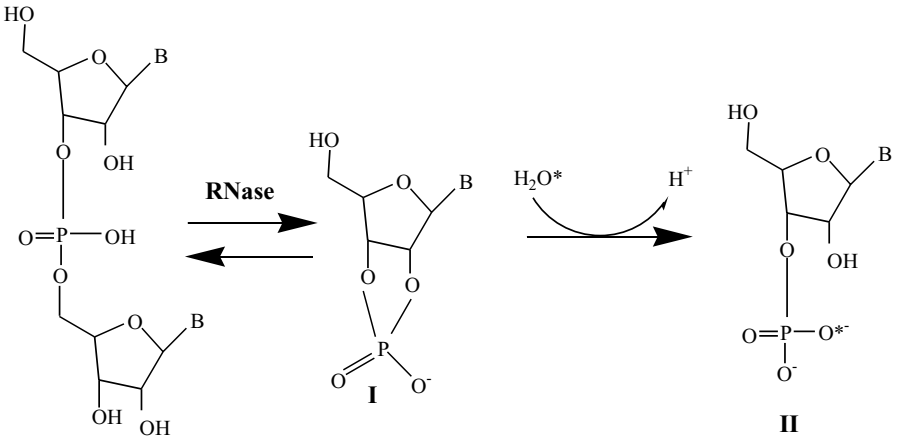

Fig. 1. Endonuclease cleavage mechanism. The 3′-cyclic phosphate (**I**) is an intermediate in this reaction. Because an oxygen from water present in the buffer is incorporated at the 3′-phosphate (**II**), a facile means of stable isotope labeling is available.

ing ESI-based collisionally induced dissociation (CID) approaches. In addition, several reports illustrate that multiply charged oligonucleotides can provide significant sequence information during low-energy CID [10,19–26].

Here, we demonstrate that the combination of ^{18}O labeling and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICRMS) is an effective approach for mapping intact RNAs and their post-transcriptional modifications. An ^{18}O label is incorporated at the 3′-phosphate terminus of oligonucleotides upon hydrolysis of RNAs with RNase T1. The ^{18}O-labeled digestion products can then be identified directly by ESI-FTICRMS without requiring prior chromatographic separation. Once ^{18}O-labeled digestion products are identified, the combination of enzyme specificity and high mass measurement accuracy [27] limit the possible base compositions thereby simplifying the RNase mapping process. Further, the use of accurate mass measurements of RNase digestion products can be performed in the absence of any additional base composition constraints to identify ^{18}O-labeled digestion products or could be used to identify contaminating RNase digestion products by searching against RNA sequence databases. Additionally, ^{18}O-labeled RNase T1 digestion products can be isolated for further sequencing by sustained off resonance irradiation (SORI)-CID. The ^{18}O label is retained on all 3′-termini containing fragment ions and is used to simplify sequence assignments.

2. Experimental

2.1. Materials

Escherichia coli tRNA^{Val} and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. RNase T1 was obtained from Roche Molecular Biochemicals (Indianapolis, IN, USA). The ^{18}O-labeled water was purchased from Isotec (Miamisburg, OH, USA). Sep-Pak C18 cartridges were obtained from Waters (Milford, MA, USA). Nanopure water (18 MΩ) was filtered using a Barnstead (Dubuque, IA, USA) Nanopure System and autoclaved before use.

2.2. Enzymatic hydrolysis of RNA

Labeling of the RNase T1 digestion products was done by first dissolving 120 μg of tRNA in a buffer of 20 mM ammonium acetate in 50% (v/v) ^{18}O-labeled water (pH 7). This solution was incubated for 1 h at 37 °C with 1500 U of RNase T1. The digestion products were then passed through a Sep-Pak C18 cartridge and eluted in 1 ml of 75% aqueous acetonitrile for rapid desalting. Unlabeled RNase T1 digestion products were generated by incubating 120 μg of tRNA with 1500 U of RNase T1 that had been desalted by use of a Sep-Pak C18 cartridge.

2.3. Mass spectrometry

All MS experiments were done using an IonSpec HiRes ESI Fourier Transform Mass Spectrometer (Irvine, California) equipped with a 4.7 T actively shielded superconducting magnet. Prior to analysis all RNase digestion products were reconstituted in 50% aqueous isopropanol and then directly infused into a tapered 30 μm i.d. fused silica micro-ESI needle at a rate of 200–500 nl min^{-1}. An ESI capillary voltage of between 1.5 and 3.0 kV was used. Ions were transferred and allowed to accumulate into the Penning ion trap (trapping voltage = −6 V) through a quadrupole ion guide operated at 2.1 MHz. Broadband excitation (105 $V_{b\text{-}p}$, T_{ex} = 4 ms) and detection (512 K data points; 2 MHz) were used for all RNase mapping experiments. The time-domain ICR signal (typically 10 scans) was subjected to baseline correction followed by apodization and zero-filling (2×) before Fourier transformation and magnitude-mode calculation. External calibration was done prior to analysis of RNase digestion products.

2.4. SORI-CID analysis

For all MS/MS experiments, the precursor ion of interest was isolated using the arbitrary waveform generator. The *m*/*z* window for isolating the ion of interest was 6 *m*/*z* units wide to include the entire isotopic envelope of the RNase T1 digestion products. After precursor ion isolation, a 4-ms pulse of nitrogen collision gas raised the background pressure in the Penning ion trap to 10^{-6} Torr. SORI of the isolated ion was done for 750 ms, and the resulting products ions were excited and detected as described above with the exception that typically 20 scans were accumulated.

3. Results and discussion

3.1. RNase mapping of $tRNA^{Val}$ by ESI-FTICRMS

ESI-MS spectra of unseparated mixtures are generally complicated due to the multiple charging effect of ESI. In addition, ESI-MS analysis of oligonucleotides is particularly sensitive to sample and solution contaminants, such as salts [28–30]. For these reasons, ESI-MS has not been previously reported for RNA modification mapping without prior on-line separation.

Fig. 2a is an illustration of the typical spectral complexity arising from the analysis of an unseparated mixture of RNase digestion products of a 76-mer RNA. Over 40 mass unique peaks were found after analysis of the data in Fig. 2a. As discussed previously by Pomerantz et al., the combination of endonuclease specificity and accurate mass measurement constrains the possible oligonucleotide compositions that should arise during RNase mapping [27]. Thus, in principle, one only needs to search the unique mass values against constrained oligonucleotide databases to identify each product. When such a protocol was used for the digestion products arising from the spectrum in Fig. 2a, only 10 of the unique mass values obtained during RNase mapping actually map to the known sequence of $tRNA^{Val}$ (cf., Table 1). Thus, while the information obtained from the mass spectral analysis of unseparated RNase digestion products is quite significant, only a limited number of the mass values lead to characterization of the initial RNA sample.

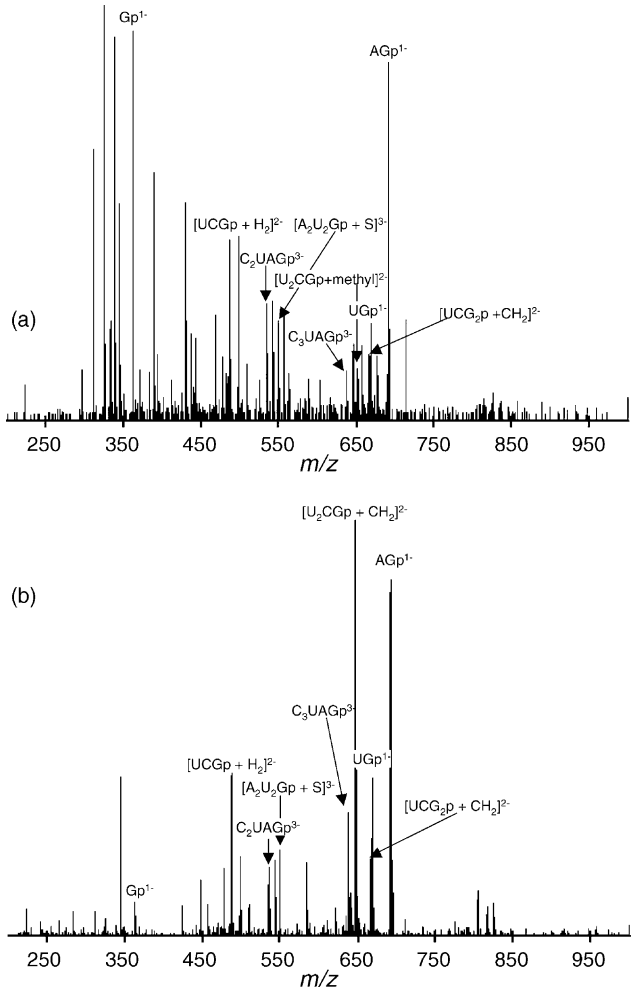

Fig. 2. ESI-FTICR mass spectral data from the RNase T1 digestion of *Escherichia coli* $tRNA^{Val}$ in (a) unlabeled water and (b) 50% (v/v) $H_2{}^{18}O$ water. The additional spectral complexity observed in spectrum (a) is due to salt adducts.

Table 1
Experimental monoisotopic molecular weights from the ^{18}O-labeled *Escherichia coli* $tRNA^{Val}$ digestion products with calculated oligonucleotide compositions generated with the constraint that each digestion product must contain 3′-Gp

M_r (^{18}O labeled)	Base composition	M_r (calculated)	Δm	$tRNA^{Val}$ sequence
363.0545	Gp	363.0579	−0.0034	G2; G3; G19; G20; G40; G43; G44; G45; G50; G53
668.1004	CGp	668.0992	0.0012	C51:G52
669.0848	UGp	669.0832	0.0016	U4:G5
692.1112	AGp	692.1105	0.0007	A21:G22; A23:G24; A41:G42
976.1312	UCGp + H_2	976.1402	−0.0090	C16:G18
1294.156	U_2CGp + methyl	1294.166	−0.010	m^5U54:G57
1333.176	UCG_2p + methyl	1333.188	−0.012	m^7G46:G49
1608.204	C_2UAGp	1608.218	−0.014	C11:G15
1649.176	A_2U_2Gp + thio	1649.191	−0.015	A6:G10
1913.260	C_3UAGp	1913.260	0.000	A58:G63

Only doublets that map onto the known $tRNA^{Val}$ sequence are included.

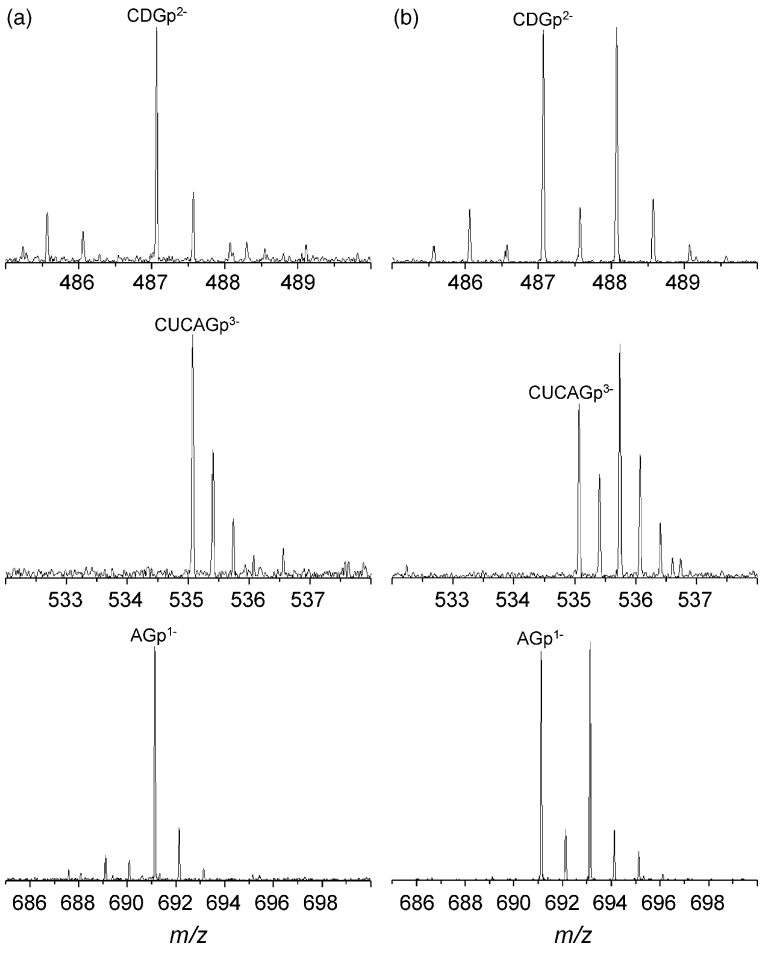

Fig. 3. ESI-FTICR mass spectra of RNase T1 digestion products obtained (a) without and (b) with ^{18}O labeling. The top panel is the doubly charged ion of CDGp, where D represents the posttranscriptionally modified residue dihydrouridine; the middle panel is the triply charged ion of CUCAGp; and the bottom panel is the singly charged ion of AGp. The isotopic label is readily distinguished in the ESI data and is used to simplify subsequent data analysis.

For simple confirmation of oligonucleotide base composition, the approach used above, while tedious, is applicable and when used in combination with LC-MS is particularly effective for the analysis of various transfer and ribosomal RNAs [3,8,9]. However, when attempting higher throughput analysis of RNA (e.g., without on-line LC-MS) or when attempting to characterize systems of greater complexity, it would be useful to introduce additional constraints into the analysis step. As demonstrated first in MALDI-based RNase mapping experiments [16], the use of ^{18}O-labeled water during the endonuclease digestion leads to characteristic "A" and "A + 2" doublets that uniquely identify 3′-phosphate digestion products. With this approach, only unique mass values exhibiting the characteristic doublet would be candidates for base composition determination, thereby reducing the data analysis demands.

Fig. 2b is a representative electrospray mass spectrum arising from the RNase T1 digestion of a tRNA done in the presence of labeled water. To illustrate the characteristic doublet features that arise due to the incorporation of the ^{18}O label during enzymatic hydrolysis, Fig. 3 shows the isotopic pattern of *E. coli* $tRNA^{Val}$ digestion products without (Fig. 3a) and with (Fig. 3b) the ^{18}O label for several different singly and multiply charged ions. Even for multiply charged ions, the presence of an "A+2" peak of nearly equal abundance to the "A" peak readily identifies the ion as arising from enzymatic hydrolysis via the scheme presented in Fig. 1. Characteristic doublets are seen at *m/z* 470, 535, and 691 in Fig. 3b, which denotes that these ions must contain a 3′-guanosine monophosphate (3′-Gp) if they arise from RNase T1 (vide infra). In such cases, therefore, only those ions containing the characteristic doublet feature would need to be analyzed and identified for the initial RNase mapping experiment.

Doublets identified from the data shown in Fig. 2b are listed in Table 1. With the restriction that each doublet must contain a 3′-Gp, base compositions of digestion products in the absence of posttranscriptionally modified nucleosides are determined easily. In the data examined here, six of the detected doublets can be assigned as unmodified oligonucleotides that contain 3′-Gp and mapped onto the tRNA sequence. Because a large majority of posttranscriptional

modifications contain conserved modification motifs (e.g., methylation) [31], these modification motif masses can be subtracted from unassigned molecular weights to provide additional information into the putative modification present. For example, subtracting 14 Da from the originally unassigned doublets at 1294/1296 and 1333/1335 lead to base compositions of U_2CGp and UCGGp, respectively. These base compositions can then be matched against the known $tRNA^{Val}$ sequence data to determine the placement of the RNase T1 digestion products in the overall sequence as noted in Table 1. In a similar fashion, the originally unassigned doublets at 976/978 and 1649/1651 were also searched using known modification masses resulting in the final mapping assignments shown in Table 1.

Through this simplified process, over 63% of the tRNA sequence can be mapped by use of the ^{18}O-labeled products alone. Because the 3′-terminus of the tRNA is known to contain a hydroxyl group, the use of ^{18}O labeling will not reveal this terminal oligonucleotide. However, working from known sequence data, the unmodified mass of the 3′-terminal RNase T1 digestion product would be 4001.6 Da and detection of this unlabeled RNase T1 digestion product increased the total amount of tRNA sequence mapped to 80%.

Even using this approach for RNase mapping, several important experimental discrepancies were noted. Complete sequence coverage of the tRNA was not obtained in any of the experiments done in this work. Only one expected oligonucleotide was not detected, a 15-mer (C25:G39). To confirm that the C18 desalting step used in this work did not lead to the loss of this large oligonucleotide from the sample mixture, MALDI-MS analysis of the same mixture was done and the 15-mer is detected in that experiment (data not shown). It is most likely that the ionization of this significantly larger oligonucleotide may be suppressed during the ESI process due to its increased hydrophobicity [32]. Future efforts at optimizing the ESI infusion buffer and FTICRMS experimental parameters are planned to increase the detection of more hydrophobic oligonucleotides in such complex mixtures. Alternatively, other endonucleases (e.g., RNase A) could be used to provide overlapping and redundant mapping information to completely characterize intact RNAs [13].

Seven of the isotopic doublets that were detected do not correspond to unmodified or posttranscriptionally modified RNase T1 digestion products of $tRNA^{Val}$ (Table 2). Four such doublets yielded base compositions containing a single 3′-Gp within the experimental error of these mass measurements, yet these base compositions do not map onto the known sequence of the tRNA used in this work. A search of the data obtained in this work against the other two known $tRNA^{Val}$ sequences also did not reveal matches to any of the unassigned digestion products. Although the tRNA used in this work was obtained commercially, it was used without further purification, so it is possible that contaminating RNAs were present.

In addition, it is assumed in this study that isotopic labeling only occurs during enzymatic processing following a mechanism illustrated in Fig. 1. As the purity of the RNase T1 used in these studies was not assessed prior to use, it is possible that the endonuclease was contaminated with other RNases, such as RNase T2. Because RNase T1, T2, A, and U2 all have the same hydrolysis mechanism, the unassigned digestion products can then searched without the constraint that the oligonucleotide must contain a 3′-Gp. Searching the unassigned digestion products without this constraint did not yield any base compositions that matched to the possible endonuclease digestion products of the tRNA used in this work. This result strongly suggests that the remaining unidentified 3′-terminal phosphate endonuclease digestion products contain less common posttranscriptional modifications. Studies characterizing these particular anomalous doublets as well as unlabeled oligoribonucleotides present in the mass spectrum are in progress.

Table 2
Experimental monoisotopic molecular weights from the ^{18}O-labeled *Escherichia coli* $tRNA^{Val}$ digestion products containing isotopic doublets that do not generate unmodified base compositions or that cannot be mapped onto the known $tRNA^{Val}$ sequence

M_r (^{18}O labeled)	Base composition	M_r (calculated)	Δm
949.1090	No match	–	–
998.1324	UAGp	998.1357	−0.0033
1021.155	A_2Gp	1021.163	−0.008
1168.119	No match	–	–
1263.193	No match	–	–
1279.180	C_2UGp	1279.166	0.014
1633.211	U_2A_2Gp	1633.214	−0.003

3.2. Effects of mass accuracy and phosphate labeling in RNase mapping

While the scheme as implemented in this work could be done using a variety of ESI-based mass spectrometers, the use of a high resolving power instrument, like the FTICR mass spectrometer, provides additional advantages when attempting to interpret peaks as endonuclease digestion products. For example, the doublet detected at 998/1000 could be either UAGp, which does not map to the known $tRNA^{Val}$ sequence, or a sodium adduct of CDGp, where D represents the posttranscriptionally modified nucleoside dihydrouridine ($U + H_2$), identified in Table 1. Although it is tempting to explain away this doublet as a sodium adduct, accurate mass analysis of these two possibilities finds that the sodium adduct has a mass error of 36 ppm, which is significantly greater than the mass accuracy of the experimental measurement. In a similar fashion, although the doublet at 1263/1265 suggests a base composition of UC_2Ap from nominal mass considerations, the mass error is 28 ppm, which is also significantly greater than the mass accuracy of the experimental measurement.

In addition to these specific examples demonstrating the usefulness of higher mass measurement accuracy for RNase mapping experiments, the combination of ^{18}O labeling and

high mass measurement accuracy of FTICRMS provide additional advantages for RNase mapping. At the 10 ppm mass measurement accuracy level available in this work, the base composition can be uniquely defined for any unmodified oligoribonucleotide up to the 5-mer level with one exception (A_5p versus G_5). Defining the presence of a 3-terminal phosphate group by use of the ^{18}O label extends the size of oligoribonucleotides whose base compositions can be uniquely defined by mass measurements at the 10 ppm level up to a 10-mer. This combination now allows one to use endonucleases with limited specificity (e.g., RNase A which cleaves preferentially on the 3′-side of U or C residues) for RNase mapping as the constraints provided by accurate mass measurement and knowledge of the 3′-phosphate compensate for the lack of knowledge about numbers of a given residue. Moreover, when the number of a particular residue is known, such as is the case when RNase T1 is used, high mass accuracy and knowledge of the 3′-phosphate lead to significant restrictions in base composition for oligoribonucleotides. In particular, for RNase T1 such constraints uniquely define the base composition for oligoribonucleotides below mass 11,000, and there are only five isobaric oligoribonucleotide compositions within 10 ppm at the 40-mer level or below (Table 3).

While it is unlikely that any naturally occurring RNAs would be that deficient in any one residue (even accounting for variable transcription, the overall relative abundance of (G + C) in the human genome is ∼40% [33]), the more likely application of this approach will be in the identification of posttranscriptionally modified nucleosides. Methylation is the most common modification motif in RNA [34]. Because any modification that increases the mass of an oligoribonucleotide, such as methylation, results in an increase in the number of possible base compositions in the absence of any compositional constraints [27], the effect of mass accuracy and ^{18}O labeling on compositional uniqueness of modified oligoribonucleotides was examined. With no residue constraints, at the 5 ppm mass measurement accuracy level, a unique base composition can be determined up to the 7-mer level for oligoribonucleotides containing as many as four methylations. Moreover, when the number of any one particular residue is constrained (e.g., G = 1 representing RNase T1 digestion products), the use of ^{18}O labeling and FTICRMS analysis at the 10 ppm mass accuracy level can uniquely identify methylated oligoribonucleotides up to the 20-mer level so long as there are no more than four methylated nucleosides per RNase digestion product. It is expected that similar trends would be observed for other less common modification motifs. Thus, the combination of mass measurement accuracy, phosphate labeling, and endonuclease specificity offer a powerful approach for directly identifying the presence of posttranscriptionally modified oligoribonucleotides.

Table 3
Isobaric oligoribonucleotides compositions within 10 ppm at the 40-mer level or below

Composition	Mass	Composition	Mass
$C_{37}Gp$	11649.585	$C_{37}U_2Gp$	12261.635
$U_{10}A_{25}Gp$	11649.623	$U_{12}A_{25}Gp$	12261.674
$C_{37}UGp$	11955.610	$C_{37}UAGp$	12284.663
$U_{11}A_{25}Gp$	11955.648	$U_{11}A_{26}Gp$	12284.701
$C_{37}AGp$	11978.637		
$U_{10}A_{26}Gp$	11978.676		

3.3. FTICRMS/MS of RNase digestion products

While RNase mapping of the type illustrated here or shown by use of MALDI-MS is useful for determining the presence of posttranscriptional modifications, placing suspected modifications within the overall sequence of the RNA is still required to fully characterize a particular nucleic acid. With ESI-FTICRMS, isolation and sequencing of suspected modified oligonucleotides by CID is feasible and easier to implement than most commercial MALDI-TOF instruments. In addition, as shown in prior MALDI-PSD sequencing of ^{18}O-labeled oligonucleotides [16], the presence of the ^{18}O label defines the 3′-termini containing fragment ions, which should simplify subsequent sequence assignments.

To verify experimentally the advantages of ^{18}O labeling in MS/MS experiments, several doublet ions generated in the initial RNase mapping experiment were selected for further analysis. Fig. 4 is representative MS/MS data obtained by SORI-CID of the doubly charged ion at *m/z* 955.6. The initial mass measurement constrained the base composition to be (C_3UA)Gp. To ensure that any 3′-terminus containing fragment ions (*w*, *x*, *y*, or *z* ions following the nomenclature of McLuckey [19]) would retain the doublet feature during MS/MS, the precursor ion selection window was set to isolate the entire isotopic envelope and not just the monoisotopic ion. Although less than optimal fragmentation was found for this oligonucleotide, analysis of those fragment

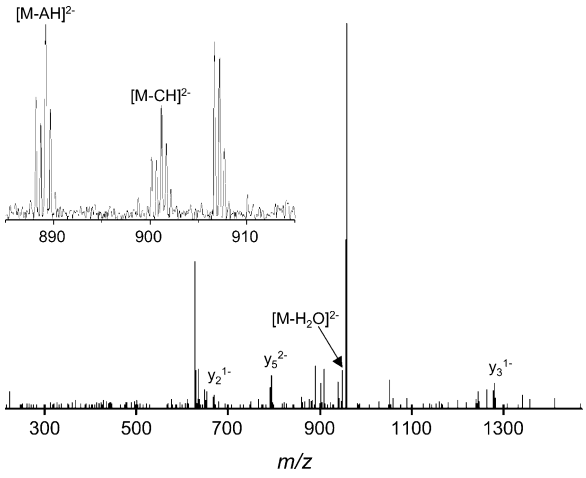

Fig. 4. ESI-FTICRMS/MS of *m/z* 955.6, the doubly charged ion of the 1913 Da digestion product, C_3UAGp. As noted in the inset, 3′-termini containing fragment ions exhibit the isotopic label from which the sequence, AUCCCGp, is readily determined (Table 4).

Table 4
Experimentally measured monoisotopic molecular weights of ^{18}O-labeled fragment ions and sequence assignments arising from SORI-CID of *m/z* 955.6

^{18}O-labeled fragments	Fragment	Oligonucleotide sequence
668.1135	y_2	CGp
1278.229	y_3	CCCGp
1584.233	y_5	UCCCGp
1762.242	M − GH	AUCCCGp − GH
1778.246	M − AH	AUCCCGp − AH
1802.258	M − CH	AUCCCGp − CH
1895.310	M − H_2O	AUCCCGp − H_2O

ions shown to contain the ^{18}O label yielded sufficient information to verify the sequence of this oligonucleotide as AUCCCGp (Table 4).

As another test, the doubly charged ion at *m/z* 823.6 was isolated and subjected to SORI-CID. As noted in Table 1, the base composition of this precursor ion corresponds to U_2A_2Gp with a 16 Da modification. This modification motif (+16 Da) occurs for thiolated nucleosides, and only uridine and cytidine are known to have single thio modifications [34]. Thus, to experimentally determine the sequence placement of the thio-modified uridine, the ^{18}O-labeled precursor isotopic envelope was isolated and subjected to MS/MS analysis. The mass spectrum resulting from the SORI-CID of this oligonucleotide is seen in Fig. 5 and those fragment ions found to contain a doublet are listed in Table 5. As can be seen by the data in Table 5, the overall sequence of the oligonucleotide was determined to be AUU*AGp with the thio modification denoted by U* in the preceding sequence. Both s^2U and s^4U are known to be present in bacteria, therefore, the exact identity of this post-transcriptional modification cannot be determined solely by

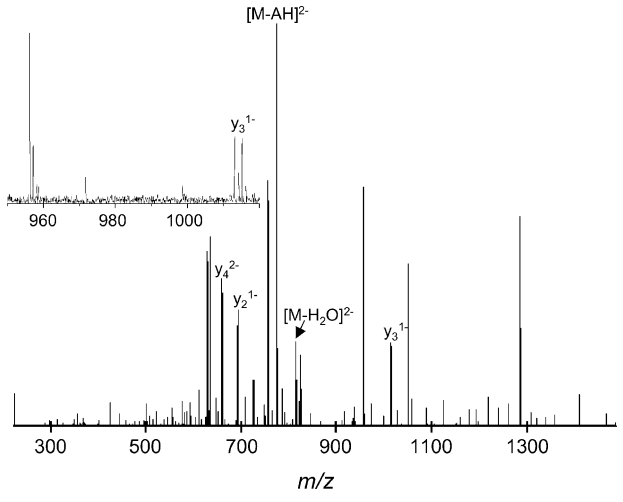

Fig. 5. ESI-FTICRMS/MS of *m/z* 823.6, the doubly charged ion of the 1649 Da digestion product, U_2A_2Gp, plus a thio modification. As in Fig. 4, the inset shows that the 3′-termini containing fragment ions exhibit the isotopic label simplifying the sequence interpretation of this modified oligonucleotide (Table 5).

Table 5
Experimentally measured monoisotopic molecular weights of ^{18}O-labeled fragment ions and sequence assignments arising from SORI-CID of *m/z* 823.6

^{18}O-labeled fragments	Fragment	Oligonucleotide sequence
692.1199	y_2	AGp
1014.143	y_3	sUAGp
1320.161	y_4	UsUAGp
1514.169	M − AH	AUsUAGp − AH
1631.212	M − H_2O	AUsUAGp − H_2O

sU denotes a thiolated uridine residue.

the MS/MS data obtained in this example. However, the method outlined here would be compatible with further ion isolation and dissociation steps (MS^n) that could possibly distinguish between these two possibilities. Alternatively, additional experiments involving LC-MS analysis of the enzymatically hydrolyzed oligoribonucleotide can be used to distinguish between the two thiolated uridine residues [35].

4. Conclusions

The incorporation of an ^{18}O label at the 3′-phosphate end of oligonucleotides upon hydrolysis of RNAs with RNase T1 has been used to simplify RNA mapping implemented with ESI-FTICRMS. ESI spectra interpretation is minimized by the easily recognizable doublet feature of the complete digestion products. The presence of the ^{18}O label was also used to facilitate assignment of sequence specific oligonucleotide fragment ions. The additional information provided by high accuracy mass measurements in combination with isotopic labeling revealed digestion products that could not be assigned to the model system used in this work. Further work at characterizing these anomalous digestion products and the use of this approach for higher throughput RNase mapping of less characterized RNAs are planned.

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