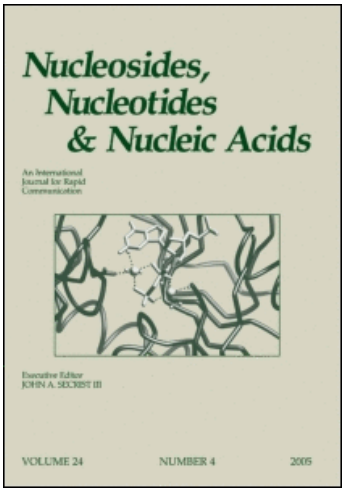


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# New Techniques for the Rapid Characterization of Oligonucleotides by Mass Spectrometry

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# NEW TECHNIQUES FOR THE RAPID CHARACTERIZATION OF OLIGONUCLEOTIDES BY MASS SPECTROMETRY

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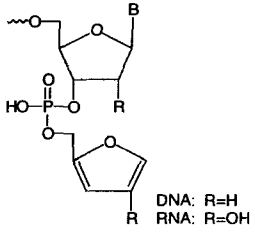
ABSTRACT: Recent advances in combined HPLC/electrospray ionization-mass spectrometry provide effective new capabilities for the rapid characterization of oligonucleotides. Accurate mass measurements with errors <0.3 Da, and determination of base and sugar modification and of nearest neighbor identities, can be routinely carried out on 10-100 component mixtures of RNA or DNA. These procedures are widely applicable in structural and analytical studies involving mixtures of oligonucleotides.



Revolutionary developments in mass spectrometry in recent years have provided new methods for the structural characterization of oligonucleotides[1]. In particular, the parallel discoveries of electrospray ionization[2] and matrix-assisted laser desorption ionization (MALDI)[3] have resulted in a new generation of instruments and protocols now coming into routine use in fields ranging from oligonucleotide synthesis to molecular biology. At the heart of these methods is the capability to transfer solution-phase polynucleotide ions to the gas phase, where they may be dissociated into information-bearing fragments,[4] or mass-measured with errors typically in the range 0.01-0.1% (e.g., ± 1–10 Da at 10 kDa). Because mass is an intrinsic molecular property, and the elements of nucleotide sequence are represented by changes in mass, mass spectrometry-based techniques are particularly effective in the characterization and sequencing of oligonucleotides modified in the base, sugar or backbone[5,6].

A notable step in the extension of these methods to mixtures of oligonucleotides has been the introduction of an HPLC buffer system based on 1,1,1,3,3,3-hexafluoro-2-propanol/triethylamine for directly combined HPLC/electrospray ionization mass

TABLE 1. Examples of electrospray ionization interface reactions for use with LC/MS of oligonucleotides.

| acquisition mode | oligonucleotide fragments formed in ionization region | MS-1 | product transmitted and detected by MS-2 | measurement |
|---|---|---|---|---|
| MS | base anion | scans the base *m/z* region | not used | base identity |
| | base side chain fragment | scans the low mass region | not used | presence of specific modification |
| |   (a – base ion series) | scans *m/z* 340–1200 | not used | sequence 5′ → 3′ |
| | –pNpNp-3′ (w ion series) | scans *m/z* 300–1200 | not used | sequence 3′ → 5′ |
| MS/MS | $N^1pN^*>p$, $N^*pN^2>p$ | selects each of four dinucleotide masses for $N^1$, $N^2$ = A,C,G,U | $N^*>p$ | nearest neighbors of $N^*$ |
| | pNm | selects each of four pNm masses for N = A,C,G,U | base anion | presence of 2′-*O*-methylribose |
| | pNm | selects each of four pNm masses for N = A,C,G,U | $PO_3^-$ | presence of 2′-*O*-methylribose |

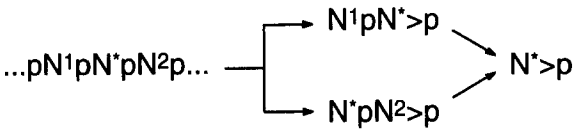



FIGURE 1. Ion dissociation pathways used for determination of the nearest neighbors of residue N*. The notation ">p" does not distinguish the 2′,3′-cyclic phosphate from various dehydrophosphate structures.

spectrometry (LC/MS) of oligonucleotides[7]. We have found this LC/MS system to be advantageous in two particular respects: first, for sensitive and rapid mass analysis of complex mixtures of oligoribonucleotides resulting from RNase T1 cleavage (at the 3' side of each Gp) of RNAs[8]: second, for design of new protocols based on fragment ions formed in the high pressure region of the electrospray ionization source (summarized in ref. 9). Examples of these measurements are given in Table 1, and can be carried out using single mass analyzer (i.e. quadrupole) instruments (MS) or those with tandem mass analyzers (MS/MS).

We view two of these approaches as potentially very useful for analysis of oligonucleotides in many types of problems: the determination of nearest neighbors, and the detection of O-2' modification (illustrated as 2'-*O*-methylribose in Table 1). Both types of measurement can be made in a single (or separate) experiment to all components of the mixtures. The principle of the nearest neighbor method[10] is shown in Fig. 1. A polynucleotide (e.g. 3–70-mer) containing the residues of interest N* dissociates in the electrospray interface to produce numerous small ions including the two N*-containing dimers shown. They in turn dissociate in the collision cell located between the two mass analyzers, to N*>p, from which the identities of the two neighbors $N^1$ and $N^2$, with their relative sequence not specified, are deduced.

The choice of pathways monitored for recognition of O-2' methylation is shown in Fig. 2, and is based on the formation of ribose-methylated mononucleotides pNm in the electrospray ionization interface[11]. Pathways 1, 2, or 3 provide selective detection of any of the four (or other) methylated residues in the polynucleotide. For instance, detection of a signal in the *m/z* 360→225 channel indicates presence of 2'-*O*-methyladenosine, while

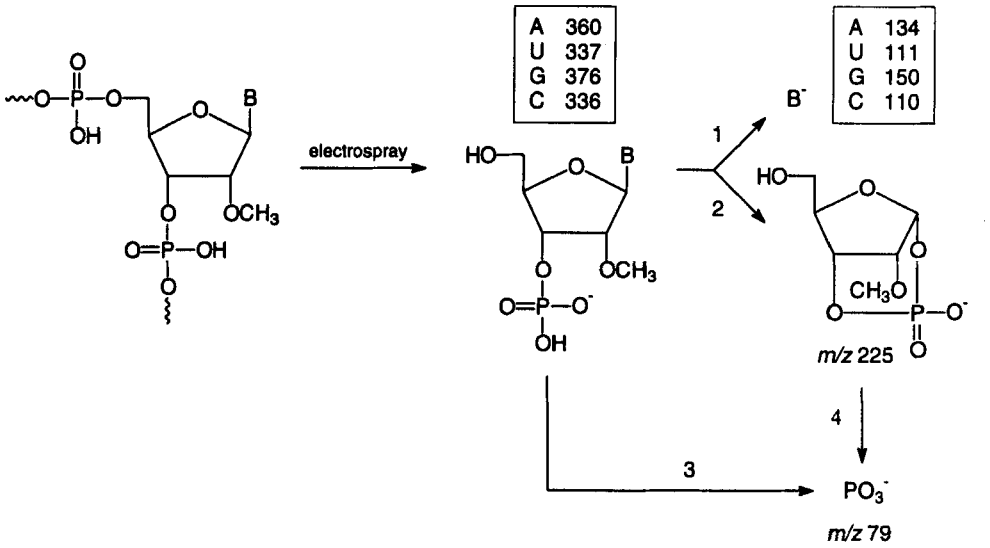



FIGURE 2. Ion dissociation pathways available for detection of 2′-O-methyl substituents in oligonucleotide mixtures.



the m/z 225→79 channel can be used for generic detection of methylated ribose, for any mass value of base B.

ACKNOWLEDGMENT

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