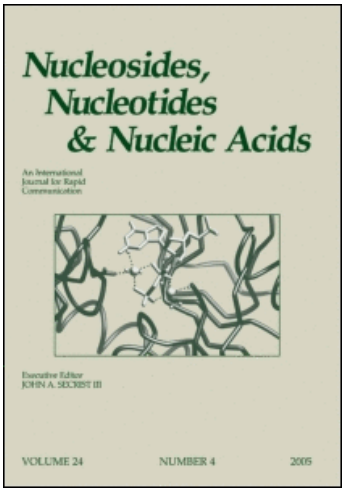

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Recent Developments in Mass Spectrometry: Rna Sequencing

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RECENT DEVELOPMENTS IN MASS SPECTROMETRY: RNA SEQUENCING

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ABSRACT: Simplified sequencing of an oligoribonucleotide containing 16 bases is accomplished by matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. We used delayed ion extraction (DE) technique and kinetic degradation with two exonucleases.

The increasing use of RNA in research and diagnostics requires a standard sequencing method for isolated or synthetic modified as well as unmodified RNA strands. Kinetic MALDI-TOF[1] exonuclease sequencing is a powerful tool for the determination of sequencing information of nucleic acids[2,3] by comparing adjacent fragments produced by our group using MS/MS experiments and nanoelectrospray techniques.[4,5,6] Since elimination reactions of the sample can occur during measurement and a dominant fragmentation series is necessary for the determination of the sequencing direction by this method we used enzymatic degradation where the direction of sequencing is defined by the enzyme used. We report the sequencing of the oligoribonucleotide 5'-OH-GCGUACAUCUUCCCCU-OH-3' by kinetic matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry using delayed ion extraction (DE) techniques.[7]

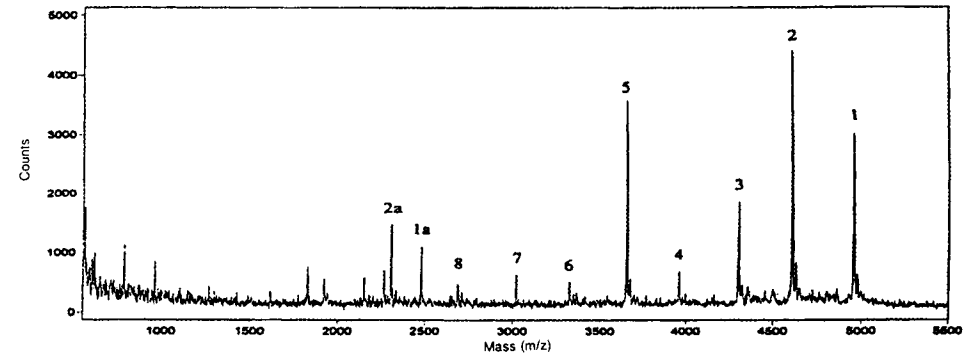
Time dependent DE-MALDI-TOF mass spectrometry was performed using a Perseptive Biosystems Voyager mass spectrometer containing an UV nitrogen laser emitting at 337 nm. Source voltage for experiments was 25000 V. Sequence of RNA strand (16 mer) was as follows: 5'- OH-GCGUACAUCUUCCCCU-OH-3'. After synthesis on an ABI Synthesizer the compound was purified by ion exchange and size exclusion chromatography. 2,4,6-trihydroxy-acetophenone/ammoniumcitrate was used as

TABLE 1: Masses and sequences of the oligoribonucleotide (Peak 1) produced by enzymatic digestion (5'→ 3' direction) with phosphodiesterase from bovine spleen (recorded with DE-MALDI)

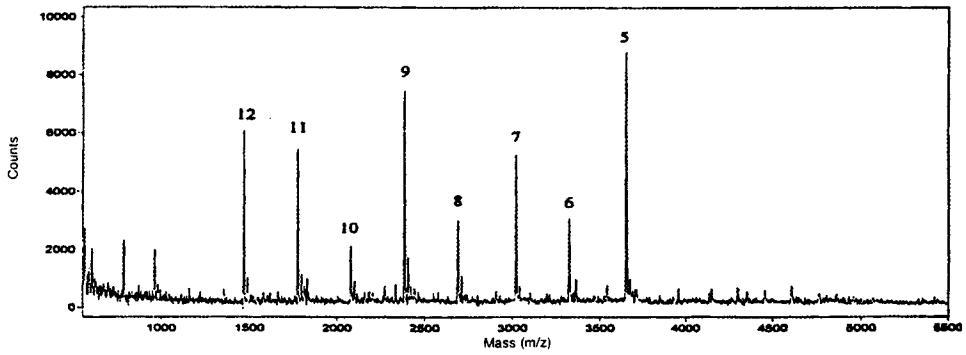
Peak	Sequence	Mass [g/mol]	Δ Mass [g/mol]
1	GCGUACAUCUUCCCCU	4954,0	/
2	CGUACAUCUUCCCCU	4608,9	345,1
3	GUACAUCUUCCCCU	4303,4	305,5
4	UACAUCUUCCCCU	3958,4	345,0
5	ACAUCUUCCCCU	3651,8	306,6
6	CAUCUUCCCCU	3322,5	329,3
7	AUCUUCCCCU	3017,3	305,2
8	UCUUCCCCU	2687,6	329,7
9	CUUCCCCU	2381,3	306,3
10	UUCCCCU	2075,8	305,5
11	UCCCCU	1769,4	306,4
12	CCCCU	1462,7	306,7

TABLE 2: Masses and sequences of the oligoribonucleotide (Peak 1) produced by enzymatic digestion (3'→ 5' direction) with phosphodiesterase from crotalus durissus (recorded with DE-MALDI)

Peak	Sequence	Mass [g/mol]	Δ Mass [g/mol]
1	GCGUACAUCUUCCCCU	4954,2	
2	GCGUACAUCUUCCCC	4648,2	306,0
3	GCGUACAUCUUCCC	4342,8	305,4
4	GCGUACAUCUUCC	4038,0	304,8
5	GCGUACAUCUUC	3733,2	304,8
6	GCGUACAUCUU	3427,9	305,3
7	GCGUACAUCU	3122,2	305,7
8	GCGUACAUC	2815,8	306,4
9	GCGUACAU	2510,4	305,4
10	GCGUACA	2204,4	306,0
11	GCGUAC	1874,9	329,5



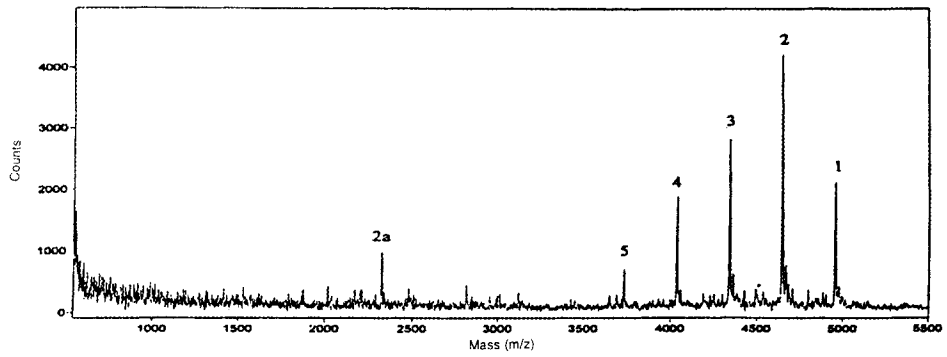
a) after 3 minutes incubation



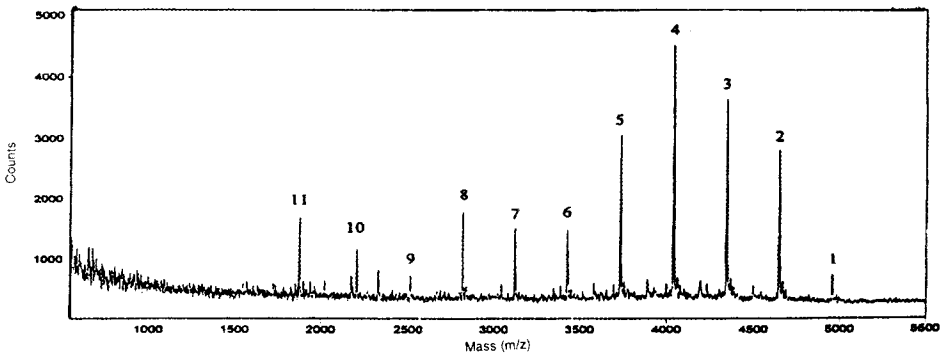
b) after 10 minutes incubation

FIG. 1: MALDI-TOF mass spectra of the RNA 16mer after digestion with 5'→ 3'-phosphodiesterase

matrix for sequencing experiments. Preparation of the matrix: Mixture 1 (2,4,6-trihydroxyacetophenone saturated in ethanol:water, 1:1) and mixture 2 (0.1 M ammoniumcitrate in water) were mixed in a 2:1 ratio. Enzymes were supplied by Boeringer Mannheim: 5'→ 3' phosphodiesterase from bovine spleen: The enzyme attacks the oligoribonucleotide 5'-OH-GCGUACAUCUUCCCCU-OH-3' at the 5' end and releases 3'-nucleotides **TABLE 1**. For 5'→ 3' digestion no additional buffer is required. 3'→ 5' phosphodiesterase from crotalus durissus: The enzyme attacks the oligoribonucleotide at the 3' end and releases 5'-nucleotides **TABLE 2**. 3'→ 5' phosphodiesterase/buffer solution: 2 µl (4 mU/µl) 3'→ 5' phosphodiesterase + 18 µl 0.1 M ammoniumcitrate pH 5.5 resulting in 20 µl 0.2 mU/µl enzyme solution.



a) after 20 minutes incubation



b) after 60 minutes incubation

FIG. 2: MALDI-TOF mass spectra of the RNA 16mer after digestion with 3'→ 5'-phosphodiesterase

Protocol of digestion procedures: 5'→ 3' direction: 740 pmol oligoribonucleotide was dissolved in 15.2 µl water and 24 mU (6 µl) enzyme buffer solution was added. Incubation was performed at 22°C. 1 µl samples were taken after an incubation time of 1, 3, 6, 10, 20 and 60 minutes (not all spectra shown) and mixed in a 1:1 ratio with the matrix. Enzymatic digestion stops when the sample are mixed with the matrix. Time dependent mass spectra of the oligoribonucleotide fragments produced by enzymatic degradation using bovin spleen 5'→ 3'-phosphodiesterase are shown in **FIG. 1**.

3'→ 5' direction: 740 pmol oligoribonucleotide was dissolved in 15.2 µl water and 0.6 mU (3 µl) enzyme buffer solution was added. Incubation was performed at 40°C. 1 µl

samples were taken after an incubation time of 1, 3, 6, 10, 20 and 60 minutes (not all spectra shown).

Time dependent mass spectra of the oligoribonucleotide fragments produced by enzymatic degradation using 3'→ 5'-phosphodiesterase are shown in **FIG. 2**.

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