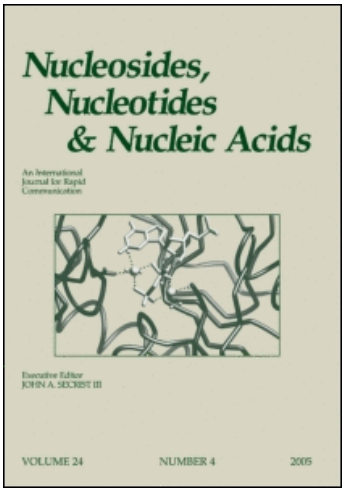

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Characterization of Oligonucleotide Sequence Isomers in Mixtures Using HPLC/MS

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CHARACTERIZATION OF OLIGONUCLEOTIDE SEQUENCE ISOMERS IN MIXTURES USING HPLC/MS

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ABSTRACT: A method is described for the analysis of mixtures containing sequence isomers of oligonucleotides. The approach consists of an electrospray ionization mass spectrometric analysis in direct combination with HPLC separation. Mass spectrometry can provide sequence information based on the fragmentation patterns of oligonucleotides allowing the simultaneous characterization of sequence isomers. An example is shown for the characterization of a mixture of dCAGT, dCGTA, dTCAG, dAGTC and dTCGA.

The identification of sequence isomers of oligonucleotides can play an important role in the analysis of combinatorial libraries containing those compounds. Mass spectrometry has shown to be a valuable tool in the sequence determination of small amounts of oligonucleotides. Recently we have discovered a method to use mass spectrometry for the analysis of nearest neighbors in oligonucleotides. [1] It is based on the fragmentation of electrosprayed molecules in the ion source of a mass spectrometer into smaller parts, including cyclic phosphate dimers ($N^1pN^2>p$) and subsequent analysis of their product ions ($N^1>p$ or $N^2>p$) by tandem mass spectrometric measurements. In direct combination with a HPLC separation, components of complex mixtures can be analyzed.

EXPERIMENTAL

Synthesis and purification of the DNA samples have been published elsewhere. [2] HPLC/ESIMS was performed on a Hewlett-Packard 1090 liquid chromatograph interfaced directly to a Fisons Quattro II mass spectrometer. Chromatographic conditions

were used as described earlier. [3] A mixture of 100 pmol of each compound was injected. For nearest neighbor measurements the cone voltage was set to 150V, the collision energy (E_{lab}) to 30eV and the dwell time to 0.2 s.

RESULTS

Ion traces of nucleotides representing 3' terminal fragments (w_1 ions) formed by so-called nozzle-skimmer dissociation in the electrospray source can distinguish four out of five oligonucleotides. Inclusion of the data obtained from the traces of the first two nucleotides from the 3' end (w_2 ions) makes it possible to identify all five tetramers. Table 1 shows the masses and retention times found for the components of the mixture.

A different approach to distinguish the individual compounds is determination of the neighbors for all nucleotides. After acquisition of selected reaction channels representing the dissociation of $N^1pN^2>p$, formed by nozzle-skimmer dissociation, into $N^1>p$ or $N^2>p$, the sequences can be deduced by the presence or absence of a signal.

TABLE 1. HPLC/ESIMS analysis of a mixture of five oligonucleotide sequence isomers

compound	ret. time (min)	M_r	3' end (w_1 ion)	3' end (w_2 ion)	nearest neighbor analysis [a]					
					CT	CA	CG	TA	TG	AG
dCAGT	14.94	1173	321	650	-	x	-	-	x	x
dCGTA	15.35	1173	330	634	-	-	x	x	x	-
dTCAG	15.56	1173	346	659	x	x	-	-	-	x
dAGTC	15.70	1173	306	610	x	-	-	-	x	x
dTCGA	15.76	1173	330	659	x	-	x	-	-	x

[a] : signals for the fragmentation of $N^1pN^2>p$ to $N^1>p$ or $N^2>p$; x: present, -: absent.

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