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Comparison of IR- and UV-matrix-assisted laser desorption/ ionization mass spectrometry of oligodeoxynucleotides

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ABSTRACT

UV-matrix assisted laser desorption/ionization mass spectrometry (UV-MALDI-MS) with 3-hydroxypicolinic acid as matrix and IR-MALDI-MS with succinic acid as matrix have proved their feasibility for highly accurate and sensitive mass determination of nucleic acids (DNA and RNA). In this work, a detailed comparison of these two MALDI-methods and between positive- and negative ion mass spectra for the analysis of oligodeoxynucleotides is undertaken. Mass spectra of DNA sequences with up to 40 nucleotides are shown. Both linear and reflectron time-of-flight mass analyzers were used within this study and are compared for their potential in the MALDI analysis of oligodeoxynucleotides. The role of molecule-ion fragmentation is also discussed.

INTRODUCTION

Within the last two years, substantial progress in the MALDI mass spectrometric analysis of nucleic acids has been achieved[1–13]. First initial studies[1,3,14–20] indicated some fundamental problems in the MALDI process of this class of analytes. A considerably lower quality of the mass spectra of oligonucleotides than typically achieved for peptides and proteins was observed, mainly characterized by a low mass resolution and a low signal-to-noise ratio. Alkali salt formation of the oligonucleotides and their desorption as molecular ions with varying number of metal cations has been revealed as one important reason for the observed peak broadening and degrading signal quality with increasing number of nucleotides of the analyte ions. This problem can be solved by replacing the metal cations by ammonium ions, which enable the formation of the free acid of the phosphodiester groups by dissociation of the corresponding ion pairs via proton transfer in the gas phase, to finally yield $(M-H)^-$- and $(M+H)^+$-ions[21,3]. A quick, simple, and efficient technique to exchange metal cations against ammonium ions within the last step of the sample preparation protocol by the addition of NH_4^+-loaded cation exchange polymer beads has been reported[3,7].

A high susceptibility of oligodeoxynucleotide ions to fragmentation, dominated by losses of nucleobases due to the labile N-glycosidic bond between the different bases and the deoxyribose moiety, strongly increasing with increasing molecular mass, has been found to be a second main problem for the analysis of nucleic acids. For MALDI we found the following orders of stability: nucleobases: T > > C > A,G, and oligonucleotides: oligothymidylic acids (and oligouridylic acids) > RNA > > DNA[7]. The mass range for which well-resolved mass spectra of oligodeoxynucleotides, containing all four different nucleotides (mixed-sequences), are obtainable, has nevertheless been extended to far above 25 nucleotides. A major improvement was achieved by the introduction of a new matrix, 3-hydroxypicolinic acid (3-HPA)[4], which proved to be highly superior for UV-MALDI of nucleic acids compared to classical protein matrices, such as nicotinic acid, ferulic acid, sinapinic acid, 2,5-dihydroxybenzoic acid, and α-cyano-4-hydroxy-cinnamic acid. 3-HPA allowed MALDI of intact molecular ions of mixed-sequence oligodeoxynucleotides with up to 67 nucleotides with a good signal-to-noise ratio, but the reported mass resolution was very low (22, FWHM). In a preceding paper[3] we have compared UV-MALDI with a laser wavelength of 337 nm using different matrices and IR-MALDI with a laser wavelength of 2.94 μm using urea and succinic acid as matrices and found the latter one to give by far the best results. In this paper, results obtained for 3-HPA as matrix using a laser wavelength of 355 nm and a reflectron time-of-flight (RTOF) instrument and using a laser wavelength of 337 nm combined with a linear time-of-flight mass spectrometer (Lin-TOF-MS) are reported and a detailed comparison between the IR- and UV-desorption schemes is given. In addition, positive- and negative-ion mass spectra of oligodeoxynucleotides are compared and discussed. Most investigations were performed on a RTOF mass spectrometer, allowing a better mass resolution and mass determination accuracy even with external calibration. A

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comparison of UV-MALDI (3-HPA) Lin-TOF- and RTOF mass spectra of DNA molecules with increasing sequence length is also given and advantages and disadvantages of the use of these two different mass analyzers for the analysis of nucleic acids are discussed.

EXPERIMENTAL

Instruments

Both MALDI-mass spectrometers used for these studies are laboratory-built instruments.

UV- and IR-MALDI-RTOF instrument[3]*:* The system is equipped with two lasers: a) an electro-optically Q-switched Er:YAG laser (1–2–3 Schwartz Electro-Optics, Orlando, FL, USA) emitting 150 ns pulses at a wavelength of 2.94 μm and b) a Q-switched frequency-tripled Nd:YAG laser (J. K. Lasers, System 2000) allowing sample irradiation at the wavelength 355 nm with a pulse duration of 7 ns. Both laser beams are focused onto the sample surface under 45° to a spot diameter of ca. 100 μm. The ions are accelerated in two stages to a total kinetic energy of 12 keV using a very low initial acceleration field strength (ca. 400 V/mm). This ion-source design reduces ion excitation by collisions within the initial very dense material plume and, thereby, keeps ion fragmentation at a low level. Ions are detected by a secondary electron multiplier (EMI 9643) equipped with a separately mounted dynode for postacceleration (ion-ion conversion). An instrumental mass resolution at up to 1000 (m/Dm, Dm: full width at the half maximum (FWHM) of the peak) can be obtained with this instrument for ions below 10,000–12,000 Da under optimal conditions. Above 15,000 Da, the mass resolution is reduced to a value of <200 due to both the ion detection process (conversion of the analyte ions to secondary-electrons and additionally to secondary-ions, strongly increasing above 10,000 Da (e.g. in Fig. 1h)) and the ion formation process.

UV-MALDI-linear-TOF instrument: A nitrogen laser at 337 nm with a pulse duration of 3 ns (VSL 337ND, Laser Science Inc. Newton, MA, USA) is focused onto the sample surface under 30° to a spot diameter of ca. 100 μm. The ions are accelerated in two stages to a total kinetic energy of 24 keV, the initial field strength is ca. 1,200 V/mm. Ion detection is done by a hybrid detector consisting of a channel plate followed by a secondary electron multiplier (EMI 9643). An instrumental mass resolution at up to 600 (FWHM) can be achieved, but typically values between 150 and 400 are obtained strongly depending on the class and size of analytes investigated and the irradiance to be applied.

On both mass spectrometers the laser irradiance is typically varied between 10^6 and 10^7 W/cm^2, the analog detector signal is digitized by a transient recorder (LeCroy 9400) at time intervals of 10 or 20 ns and further data processing is done on a PC-AT using in-house designed software. Generally, single shot spectra clearly show the molecular ion signals. To improve the signal-to-noise ratio, 10 to 20 single shot spectra were usually accumulated and averaged.

Sample preparation

Matrices. Both matrices were purified by a separate desalting step. Alkali ions were exchanged against H^+: a) on an acid (0.1 M HCl (p.a.) solution) activated cation exchange column in case of the succinic acid solution, b) on an ammonium acetate (p.a., saturated solution) loaded cation exchange column in case of the 3-hydroxypicolinic acid solution (cation exchange polymer: BioRad, 50W-X8, mesh size 100–200 μm). After lyophilizing, both matrices were dissolved in ultra pure water to a concentration of a) ca. 170 mM for the succinic acid matrix solution and b) ca. 300 mM for the 3-hydroxypicolinic acid matrix solution, aliquoted in 50 μl portions and stored at −20°C. To reduce salt contaminations by repeated handling (alkali salt traces on the pipette tips, etc.), a new portion was used every day.

Oligodeoxynucleotides: The oligodeoxynucleotides were synthesized and deprotected by Dr Otto Dahl, University of Copenhagen, Denmark.

Prior to mass spectrometry, the oligodeoxynucleotides were purified on the Pharmacia SMART™ FPLC system using a reversed phase column (C2/C18 SC2/10):

Buffer A: 5% acetonitrile/10 mM triethylammonium acetate (TEAA)
Buffer B: 30% acetonitrile/10 mM TEAA
Gradient: 0–5 min: 100% A. 5–30 min: 0–100% B. 30–35 min: 100% B. 35–40 min: 100–0% B. 40–45 min: 100% A
Flow rate: 50 μl/min

Sample preparation for MALDI-MS

Samples were lyophilized over night to remove excess ammonium salts and redissolved in ultra pure water to a final concentration of 0.01 g/l. Aliquots of 1 μl of analyte solution and 2 μl of matrix solution were mixed on a flat metallic sample support and dried in a stream of cold air. Remaining alkali cations present in the sample solution and on the sample support surface were removed from the sample droplet with NH_4^+- loaded cation exchange polymer beads as previously reported[3,7].

RESULTS and DISCUSSION

Comparison of UV- and IR-MALDI using 3-hydroxypicolinic acid and succinic acid as matrices

Sample preparation, measurement conditions and general characteristics of IR-MALDI-RTOF-MS of DNA and RNA molecules using succinic acid as matrix (IR-MALDI (SA)) have been reported earlier[3,7]. In agreement with the results reported by other groups[1,2,4,9–13,15,19,20] we found oligodeoxynucleotides to generate stronger signals and better resolution in the negative-ion mode than in the positive-ion mode. Sample preparation for UV-MALDI with the 3-HPA matrix (UV-MALDI (3-HPA)) has been investigated intensively and we found a nearly saturated aqueous solution of the 3-HPA matrix (ca. 50 g/l) to give best results on our instrument. All other preparation conditions (pipetted volumes of matrix- and analyte solution, analyte concentration, amount of added cation exchange material, and drying procedure) were the same as used for IR-MALDI (SA). The higher molar excess of the 3-HPA (matrix/analyte : 140,000 : 1) versus SA (20 g/l, 70,000 : 1) for the sample preparation indicates a slightly increased sensitivity for UV-MALDI (3-HPA). In contrast to IR-MALDI (SA), UV-MALDI (3-HPA) yielded comparable signal intensities (signal-to-noise ratio) and mass resolutions in the negative ion detection mode and in the positive one. Until now, mainly negative-ion mass spectra of this class of analyte molecules have been reported for MALDI-MS as well as for FAB-MS[22,23] and ESI-MS[21,24]. Their prominent formation was attributed to the strong anionic character of the nucleic acids.

As reported by Wu et. al.[4] both the ion yield and the quality of the mass spectra show high fluctuations from spot to spot for the 3-HPA matrix. This, however, can be influenced to some extent by varying the amount of ammonium salts present in the sample, ready for measurement. Usually ammonium salts (ammonium acetate) are present in the samples, originating from the HPLC purification step (TEAA) and from the cation exchange during the sample preparation (NH_4^+-ions). If the amount of

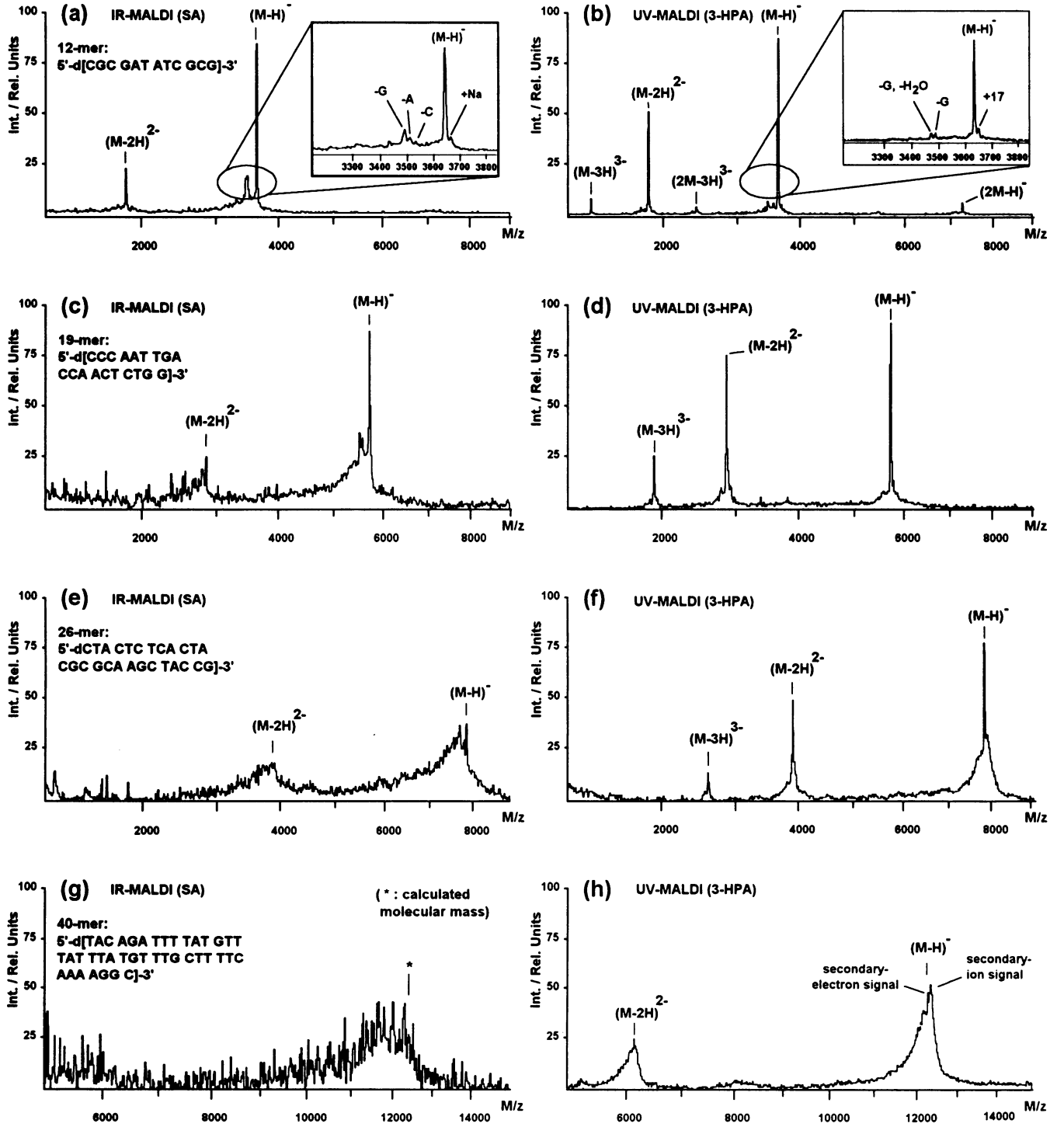

Figure 1. Comparison of negative-ion RTOF-mass spectra of oligodeoxynucleotides with increasing sequence length (12 to 40 deoxynucleotides) obtained with IR-MALDI at 2.94 μm laser wavelength and succinic acid as matrix (**a**, **c**, **e**, and **g**) and with UV-MALDI at 355 nm laser wavelength and 3-hydroxypicolinic acid as matrix (**b**, **d**, **f**, and **h**). In the mass spectrum h, additionally to the secondary-electron-signal of the SEM, a second detector-signal (secondary-ion-signal) is observed and assigned, due to the detection process of higher masses; further details are given in the experimental section. Total oligodeoxynucleotide load for each measurement: 5 pmol. All spectra are the sums of ten single-shot spectra.

ammonium salt in the prepared sample is reduced to a minimum (e.g. by previous intensive evaporation of any ammonium salt excess in vacuum from the oligonucleotide samples), very clear crystals of the 3-HPA are formed offering superior desorption conditions and allowing good mass resolution and high reproducibility for up to typically fifty shots. However, the fluctuation from spot to spot is very high; on the average, only every tenth of the crystals gives a good response. Excess of ammonium salts, on the other hand, leads to opaque crystals of 3-HPA, accompanied by white salt crystals, which reduces the local variation but results in the need for higher laser irradiance and a reduced signal-to-noise ratio as well as a lower mass resolution. We found a concentration of 1 to 10 mM ammonium acetate in the final sample droplet to form a good compromise, reducing local dependence and yielding a good signal-to-noise ratio as well as mass resolution.

Figure 1 gives a detailed comparison of negative-ion-RTOF mass spectra obtained from synthetic oligodeoxynucleotides with increasing number of nucleotides for both examined wavelength/matrix combinations. In Fig. 1a and 1b the corresponding IR- and UV-MALDI mass spectra of a DNA 12-mer are shown, demonstrating a comparable spectrum quality. A mass resolution of ca. 800 (FWHM) was measured in both cases as a typical and reproducible value. The UV-MALDI (3-HPA) mass spectrum (Fig. 1b) shows strong peaks of the doubly and of the triply charged analyte ion as well as multimers of varying charge state with lower intensities. In contrast to this, the IR-MALDI (SA) spectrum shows only the singly- and doubly-deprotonated analyte ions. The high abundance of the multiply charged oligodeoxynucleotides is a general characteristic of negative-ion UV-MALDI (3-HPA) mass spectra and is also clearly visible in Fig. 1d, f, h. We have observed that the ratio between the singly- and the multiply charged ion species is highly dependent on the desorption spot and appears to vary slightly with the analyte concentration; increasing concentration leads generally to a reduced abundance of the multiply charged species. An influence of the size of the oligodeoxynucleotides, which may have be expected due to the introduction of one strong acidic phosphodiester group for every attached nucleotide, was not observed.

As has been discussed in a previous article[7], prominent loss of the purine bases guanine and adenine and less pronounced for the pyrimidine base cytosine are the dominant fragmentation reactions in IR-MALDI (SA) (see also the extended mass window in Fig. 1a). The mass differences measured for the loss of a single base correspond to the molecular mass of the corresponding free base (111 Da for C, 135 Da for A, and 151 Da for G). The loss of thymine has never been observed. In UV-MALDI (3-HPA), base loss is also a preferred fragmentation, as has also been reported by Stemmler *et al.* for UV-MALDI (3-HPA)-ICR-FTMS[6]. Besides elimination of the nucleobases A, G, and C, often an additional loss of 18 Da is observed (see extended mass window of Fig. 1b), which most likely originates from the elimination of a water molecule after the base has been cleaved of; e.g. the 1,2-elimination of the nucleobase at the 3′-end and the successive 1,2-elimination of the 3′-hydroxyl group lead to the formation of a stable aromatic furan ring at the 3′-end. At the 5′-end, the combination of base loss and 1,2-elimination of the 5′-hydroxyl group would also generate a conjugated unsaturated system, less stable than the aromatic one, but could be converted to the furan ring by a simple following 1,3-hydrogen rearrangement. Furthermore, the loss of a single deoxynucleoside

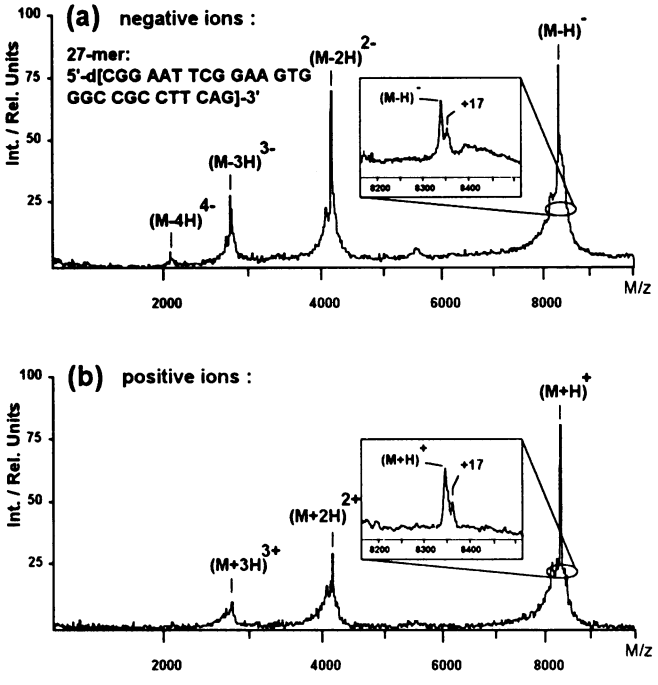

Figure 2. (a) Negative-ion UV-MALDI-RTOF and (b) positive-ion mass spectrum of a DNA 27-mer obtained with 3-hydroxypicolinic acid as matrix at 355 nm laser wavelength. Total oligodeoxynucleotide load: 5 pmol. Both spectra are the sums of ten single-shot spectra.

from the 5′-end and/or of a single deoxynucleotide from the 3′-end is often seen in the UV- and IR-MALDI mass spectra of oligonucleotides. An interesting difference between IR-MALDI (SA) and UV-MALDI (3-HPA) is indicated in the magnified part of the spectra 1a and 1b. Even if alkali ions are exchanged against ammonium ions very carefully during the sample preparation, some traces often remain in the sample, sufficient to generate a small peak of the monosodium salt of the analyte ion in IR-MALDI (SA). In UV-MALDI (3-HPA) a signal of comparable intensity, originating from the attachment of 17 Da, presumably representing the monoammonium salt of the oligodeoxynucleotide ion, has always been observed; alkali salt formation is, however, no longer detectable. The intensity of the +17-Da signal increases slightly with the molecular mass of the detected ions (see Fig. 1b, 2a, and 2b), but does not reach the signal intensity of the singly deprotonated free acid of the oligodeoxynucleotide for any sample examined up to now. The intensity of the +17-Da signal is not depending on the amount of ammonium salts present in the sample, on the laser irradiance, or on the sequence of the analyte molecule. If the assumption is true, that the +17-Da signal in the UV-MALDI (3-HPA) mass spectra originates from one undissociated ammonium phosphodiester ion pair, this can be taken as an indication for a very soft desorption/ionization process. Consequently, a too soft desorption process could lead to an excessive ammonium counterion distribution resulting in peak tailing to higher masses for bigger analyte ions instead of the peak tailing to the lower mass side caused by molecule-ion fragmentation.

In IR-MALDI (SA) the quality of the mass spectra drops dramatically with increasing molecular mass of the oligodeoxynucleotides. The signal-to-noise ratio decreases while the fragmentation increases, leading to a limited sequence length of the DNA molecules above which the intact molecular ion signal

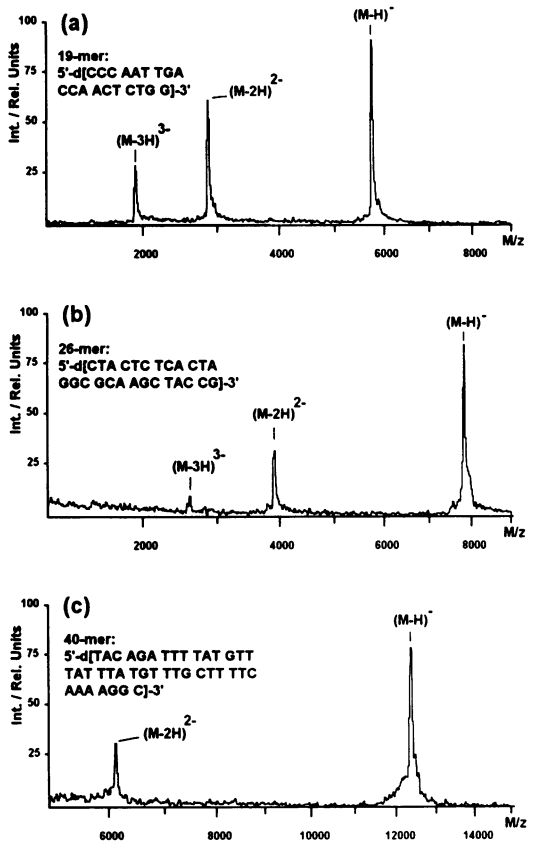

Figure 3. Negative-ion UV-MALDI-Lin-TOF mass spectra of oligodeoxynucleotides with increasing sequence length ((**a**) to (**c**) 19, 26, and 40 deoxynucleotides, respectively) obtained with 3-hydroxypicolinic acid as matrix at 337 nm laser wavelength. Total oligodeoxynucleotide load for each measurement: 5 pmol. All spectra are the sums of ten single-shot spectra.

cannot be resolved any longer. The spectrum of the DNA 26-mer (Fig. 1e) clearly demonstrates the upper limit for IR-MALDI (SA) up to which the $(M-H)^-$-signal can be resolved and an accurate mass determination is still possible. More detailed investigations on a number of oligodeoxynucleotides of varying size and sequence (12, 13, 15–20, 22, 24, 26, 27, 30, 34, 40, and 45 nucleotides) have shown that single and multiple base losses quickly increase with every additional nucleotide attached to a given sequence. Beside prompt decays, metastable decays are observed, resulting in broad unresolved fragment-ion peaks for bigger DNA-molecules (peak tailing to smaller masses). In addition to these fragmentations, prompt specific cleavages of the deoxyribose phosphate backbone occur with minor abundance which appear as well resolved smaller peaks in the middle and lower mass range of Fig. 1c and 1e. This fragmentation is strongly depending on the desorption conditions and can be significantly enhanced if the applied laser irradiance is increased. The resulting prompt fragment ions can be assigned to three series allowing bidirectional sequencing of oligodeoxynucleotides, more than 20 deoxynucleotides long, at the low pmol-level. These results will be published in a separate paper.

The mass spectrum of the DNA 40-mer (Fig. 1g) demonstrates very clearly the current limit of IR-MALDI (SA) to DNA samples. Only a very noisy, broad and unresolved peak is detected. As indicated in the spectrum by the assignment of the calculated mass of the intact analyte ion, the peak centroid is shifted to lower masses, leading to a systematically faulty mass determination. In the corresponding UV-MALDI (3-HPA) mass spectra, a much better signal-to-noise ratio is obtained, the fragmentation is reduced, and no prompt cleavages of the deoxyribose phosphate backbone are observed. As a result the accessible mass range is considerably extended; the UV-MALDI spectrum of the DNA 40-mer (Fig. 1h), for example, is comparable in quality to the IR-MALDI spectrum of the DNA 26-mer (Fig. 1e).

Comparison of negative- and positive-ion mass spectra obtained with UV-MALDI and 3-hydroxypicolinic acid as matrix

Taking the chemical structure and the very strong acidity of the nucleic acids into consideration, it seems reasonable that most of the mass spectra reported up to now for MALDI-MS, for this class of molecules, are negative-ion spectra. This agrees well with the observation made for oligodeoxynucleotide ions either generated by IR-MALDI (succinic acid or urea as matrices) or by UV-MALDI with the most common matrices (2,5-dihydroxybenzoic acid, sinapinic acid, ferulic acid, α-cyano-4-hydroxycinnamic, and nicotinic acid). Usually, considerably lower signal-to-noise ratios and a higher rate of fragmentation are found for the positive ion detection mode. In contrast to this, 3-HPA allows the acquisition of negative-ion, as well as positive-ion mass spectra of oligodeoxynucleotides of comparable quality. Figure 2 presents spectra of both ion polarities for a DNA 27-mer; the desorption was carried out from the same sample and the same spot; equivalent conditions for the ion separation and detection were used. In both spectra, not only, the signal-to-noise ratio is comparable but also the mass resolution of ca. 600 (FWHM) for both molecular ion peaks, and the abundance of the +17-Da signal (see the magnified sections of Fig 2a and b). Moreover, the peak tailing demonstrates that no significant differences exist between the two ion detection modes. The only significant difference is the reduced formation of multiply-positively-charged ion species. This may be taken as an indication for a higher ionization yield for negatively-charged ions.

For the analysis of complex mixtures of oligonucleotides (DNA or RNA[7]), e.g. oligonucleotide ladders generated in a 'Sanger'[25]- or 'Maxam–Gilbert'[26]-sequencing reaction or by an exonuclease (3'- or 5'-specific) degradation, the formation of abundant multiply-charged molecule ions causes problems, due to overlaying signals of singly-charged molecule ions of lower mass and of multiply-charged species of higher masses. Therefore, the positive-ion detection mode will be advantageous for such approaches.

Comparison of reflectron- and linear-time-of-flight mass spectra obtained with UV-MALDI and 3-hydroxypicolinic acid as matrix

As it is clearly to be seen in Fig. 1, metastable fragmentation (delayed decomposition of the molecule ions) degrades the signal resolution in the RTOF-mass spectra of oligodeoxynucleotides and prevents exact mass determination as soon as the molecule-ion signal cannot sufficiently be resolved from small mass losses. In Lin-TOF mass spectrometers, fragment ions formed by metastable decays in the field-free drift region are not separated from the intact molecule ions, thus only fragmentation occurring in the very early stage of the ion acceleration is detected as unresolved broad fragment-ion signals. Fig. 3 shows the corresponding negative-ion UV-MALDI (3-HPA) Lin-TOF mass

spectra of the DNA 19-mer, 26-mer, and 40-mer, already given as RTOF-data in Fig. 1d, f, and h, respectively. The slight difference of the used laser wavelengths (337 instead of 355 nm) for the LIN-TOF- and RTOF-measurements is due to the different types of used lasers in the two instruments (see experimental section) and does not cause any significant differences for the MALDI-process. The mass resolution in the spectra 3a, b and c various between 180 and 200 (FWHM). This is a reduction by a factor of ca. four of the RTOF-values determined for the DNA 12-mer, 19-mer, and 26-mer. In contrast to this, in the case of the DNA 40-mer, the peak width obtained in the Lin-TOF spectrum is superior due to the fact that in the RTOF-spectrum the molecule-ion signal is not sufficiently resolved from the metastable mass losses (10–15% resolution). The observation that, also in the linear-TOF spectra, molecule-ion-peak tailing to lower masses occurs, which is clearly demonstrated by Fig. 3c, indicates that with increasing size of the oligodeoxynucleotide ions more and more fragmentation takes place in the ion source region (shorter molecule-ion life-times). Thus, the problem of molecule-ion fragmentation is not overcome by using Lin-TOF instruments but it has been shifted to higher masses. From these results, one can conclude that also in Lin-TOF mass spectrometers fragmentation limits the accessible mass range for larger DNA-molecules (>>40 deoxynucleotides) in MALDI-MS.

In conclusion, for a further significant progress, a softer desorption of the nucleic-acid ions is needed; this may be reached by the advent of a new matrix or matrix/laser-wavelength combination. It has, however, to be considered that under such conditions adduct-ion formation or uncompleted dissociation of ammonium phosphodiester ion pairs may become more prominent and may replace the problem of ion fragmentation (peak tailing to higher masses instead of lower masses). Therefore, a fundamental limitation in the MALDI analysis of DNA cannot be excluded. As has been reported previously, RNA molecule ions are much more stable than DNA molecule ions in MALDI-MS (IR-MALDI spectra of RNA transcripts with more than 100 ribonucleotides were reported)[7]; thus this problem is less relevant for RNA.

The presented results document, that considerable progress can be achieved by careful sample preparation and choice of adequate ion source conditions and mass analyzer configuration, allowing highly accurate mass spectrometric analysis of larger oligodeoxy-nucleotides. In a previous paper[7] we have reported, that IR-MALDI (SA) is superior than UV-MALDI in combination with the classical peptide and protein matrices, as e.g. nicotinic acid, 2,5-dihydroxybenzoic acid, sinapinic acid and ferulic acid, for the mass spectrometricaly characterization of oligonucleotides. The data reported in this work clearly demonstrate, that using 3-hydroxypicolinic acid as matrix for UV-MALDI is actually the best approach for mass determination of nucleic acid oligomers. This is of high practical interest, because MALDI mass spectrometers basing on UV-lasers are cheaper and, more important, they are in contrast to IR-MALDI instruments commercially available. Especially Er:YAG IR-lasers are not yet routinely available and still very expensive. However, for structure analysis of oligonucleotides, prompt fragment-ion formation, observed for IR-MALDI (SA) has some promise for future applications; this will be reported in a separate paper.

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ABBREVIATIONS

ESI-MS: electrospray ionization mass spectrometry
FAB-MS: fast atom bombardment mass spectrometry
3-HPA: 3-hydroxypicolinic acid
ICR-FTMS: secondary-electron multiplier
TEAA: triethylammonium acetate
TOF: time-of-flight
UV-MALDI (3-HPA): ultraviolet matrix assisted laser desorption/ionization with 3-hydroxypicolinic acid as matrix

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