Nonresonant MALDI of Oligonucleotides: Mechanism of Ion Desorption

ARTICLE *in* ANALYTICAL CHEMISTRY · MARCH 2001

Impact Factor: 5.64 · DOI: 10.1021/ac001006+ · Source: PubMed

6 AUTHORS, INCLUDING:

Steve Allman
Oak Ridge National Laboratory
85 PUBLICATIONS **1,467** CITATIONS

SEE PROFILE

Anal. Chem. **2001,** *73,* 809−812

Nonresonant MALDI of Oligonucleotides: Mechanism of Ion Desorption

V. V. Golovlev,[†] S. H. Lee,[‡] S. L. Allman, N. I. Taranenko,[†] N. R. Isola,[†] and C. H. Chen*

Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831-6378

Oligonucleotide ions have been detected using matrix-assisted laser desorption/ionization (MALDI) under nonresonant laser irradiation of the sample. When mass resolution was not limited by adduct attachment to the analyte ions, the nonresonant MALDI spectra demonstrated better resolution than the spectra acquired with resonant ultraviolet irradiation. We found that preparation of thin-film samples on absorbing substrate surfaces was critical for the success of NR-MALDI. The possible acoustic mechanisms of ion formation and desorption are discussed.

During the past decade, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has become an important tool for accurate and fast analysis of biomolecules.[1−4] Currently, analysis of DNA fragments of 100 base pairs can be routinely performed. However, there is continued demand for improving MALDI to analyze longer DNA fragments with better spectral resolution for large-scale sequencing for research and clinical applications. Recently, an important development of the MALDI technique was introduced by using infrared (IR) laser sources for soft desorption of large oligonucleotides. It was shown that IR radiation can evaporate sample material without overexciting molecules, thus preventing postexcitation fragmentation of the analyte. By using IR-MALDI excitation, detection of DNA fragments exceeding 2000 nucleotides has been reported.[5] Although an IR source helps to reduce overexcitation of analyte molecules, it does not prevent the wide spread of ion kinetic energy. The energy spread can dramatically reduce the spectral resolution of the large molecular fragments, and consequently, reflectron time-of-flight mass spectrometry is often required to exploit the full benefits of IR-MALDI.

Investigation of the mechanism of IR-MALDI by a number of research groups has provided substantial information on this phenomenon, although it may also raise more questions. It was reported that the correlation between optical absorption and MALDI performance is not as straightforward for IR MALDI.[6,7] For a number of matrixes investigated, the wavelength could not always be assigned to specific IR absorption bands of the matrix. For some matrixes, such as succinic acid, the optimal wavelength of IR excitation was shifted 600 nm away from the matrix absorption peaks.[7] Similar experimental observation with other matrixes gave full justification to systematically investigate MALDI at conditions not traditionally considered as optimal for MALDI. In this work, our primary objective was to explore experimental conditions under which MALDI with the lowest possible number of matrix molecules transferred into gas phase could be accomplished. By reducing the number of gas-phase collisions of analyte and matrix molecules, it might be possible to reduce the energy spread of the analyte ions and thus improve spectral resolution.

We have investigated nonresonant (NR)-MALDI to follow up this concept. We believe NR-MALDI can provide the benefit of IR excitation for preventing analyte overexcitation and, due to less efficient evaporation of the matrix by laser, also reduce the energy spread of ions. With this approach, an oligonucleotide sample was crystallized with matrix on a substrate surface and irradiated by a laser beam with the wavelength off the resonance absorption of the matrix molecules. Nevertheless, we found that oligonucleotides could be detected under certain experimental conditions. Although the mechanism of NR-MALDI is not completely understood at this moment, we believe the desorption of analyte ions might be caused by acoustic waves generated in the sample substrate. Recently we demonstrated the possibility of using laser-induced acoustic waves for the desorption of small ions and proteins from a surface.[8] We believe the experimental configuration used in this work provides acoustic pulses with higher amplitude than the backside irradiation scheme explored in our previous work.[8,9] With this modification, and using matrixes for oligonucleotide ionization, laser-induced acoustic desorption can provide new options for mass spectrometry of DNA with improved spectral resolution as compared with conventional MALDI conditions.

[†] Oak Ridge Associated Universities postdoctoral fellow.

[‡] Oak Ridge Associated Universities undergraduate student.

(1) Karas, M.; Bachman, D.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 53.

(2) Tang, K.; Taranenko, N. I.; Allman, S. L.; Chang, L. Y.; Chen, C. H. *Rapid Comm. Mass Spectrom.* **1994**, *8*, 727.

(3) Taranenko, N. I.; Chung, C. N.; Zhu, Y. F.; Allman, S. L.; Golovlev, V. V.; Isola, N. R.; Martin, S. A.; Haff, L. A.; Chen, C. H. *Rapid Commun. Mass Spectrom.* **1996**, *11*, 386.

(4) Bahr, U.; Deppe, A.; Karas, M.; Hillenkamp, F. *Anal. Chem.* **1992**, *64*, 2866.

(5) Berkenkamp, S.; Kirpekar, F.; Hillenkamp, F. *Science* **1998**, *281*, 260.

(6) Cramer, R.; Haglund, R. F. Jr.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Processes* **1997**, *51*, 169−170.

(7) Sheffer, J. D.; Murray, K. K. *Rapid Commun. Mass. Spectrom.* **1998**, *12*, 1685.

(8) Golovlev, V. V.; Allman, S. L.; Garrett, W. R.; Taranenko, N. I.; Chen, C. H. *Int. J. Mass Spectrom. Ion Processes* **1997**, *69*, 169−170.

(9) Golovlev, V. V.; Allman, S. L.; Garrett, W. R.; Chen, C. H. *Appl. Phys. Lett.* **1997**, *71* (6), 852.

10.1021/ac001006+ CCC: $20.00
Published on Web 01/11/2001

EXPERIMENTAL SECTION

A few modifications of the technique for the preparation of DNA samples for NR-MALDI were explored in this work. However, the general approach shares many common procedures with regular MALDI.[8,9] 3-Hydroxypicolinic acid (3-HPA; dissolved in water−acetonitrile solution) and picolinic acid (PA; dissolved in water)[9−11] were used as matrixes of choice for these experiments. Single-stranded synthetic oligonucleotide (25-mer, M = 7620 Da, 0.2-μm synthesis scale, desalted) was purchased from Oligos Etc. and used without further purification. Samples with mixtures of different sizes of oligonucleotide were prepared using pUC 18 *Hae*III digest purchased from Sigma (SIGMA, Product No. D6293). Samples of synthetic oligonucleotide or oligonucleotide mixtures (oligos) were prepared by the following procedures. Lyophilized oligos were resuspended in sterile deionized water shortly before preparing the probe. Samples were prepared by mixing 2.5 μL of matrix solution which contained 0.2 mg of matrix (either PA or 3-HPA) and 2.5 μL of 12 μM DNA solution directly on a sample plate and air-dried at room temperature. Stainless steel plates, silicon wafers, quartz, and glass plates were used as substrates. Typically, when a drop of sample solution was deposited on the surface, it filled an area with a diameter of 4 mm. After the solution was dried, the matrix crystallized in a spot of the same diameter as the original drop of solution. The solid substance had a polycrystalline structure which is clearly visible under microscope. The thickness of the matrix layer in the central area of the spot of ~10 μm was estimated from the known weight (0.2 mg) of the matrix deposited on the surface; the size of the spot was 0.12 cm^2, and the density of the solid matrix was 2 g/cm^3.

Mass spectra from various samples were obtained using 355-, 532-, and 1064-nm radiation generated by a DC-2 Quanta-Ray Nd:YAG laser equipped with nonlinear crystals for harmonic generation. Laser pulse duration was 5 ns. The laser beam was telescoped down to a size of 500 μm × 1500 μm on the target surface. Laser intensity was tuned in the range of 10−100 mJ/cm^2 by introducing optical density filters into the laser beam and by changing the pumping energy. Mass spectra were recorded using a homemade linear TOF mass spectrometer. Pressure in the chamber of the mass spectrometer was 5×10^{-7} Torr. Positive or negative 24-kV accelerating voltage was applied to detect positive or negative ions, respectively. A microsphere plate (MSP) detector (R. M. Jordan Co.) was used for detection of ions passing through the TOF drift tube. The output electronic signal was digitized by a Yokogawa DL 2140B digital oscilloscope and transferred to a laboratory computer (PC) for further analysis.

RESULTS

There was no significant linear absorption at 532 and 1064 nm for the matrixes used in this work. Nevertheless, oligonucleotides could be observed in mass spectra acquired from the samples on a metal substrate when an appropriate procedure of sample preparation was used. For reference, we first acquired an ion signal from the 25-mer nucleotide with regular UV-MALDI conditions. A mass spectrum of positive ions desorbed by 355-nm laser beam at fluence of 40 mJ/cm^2 is shown in Figure 1A. A sample was prepared by mixing the matrix solution and DNA solution directly on the plate following the procedures described in the Experimental Section. The broadening of the oligonucleotide peak in Figure 1A is due to the ion energy spread and the attachment of sodium ions to the oligonucleotides. The mass spectra in Figure 1 were acquired using the mass spectrometer not equipped with a delayed pulse extraction.

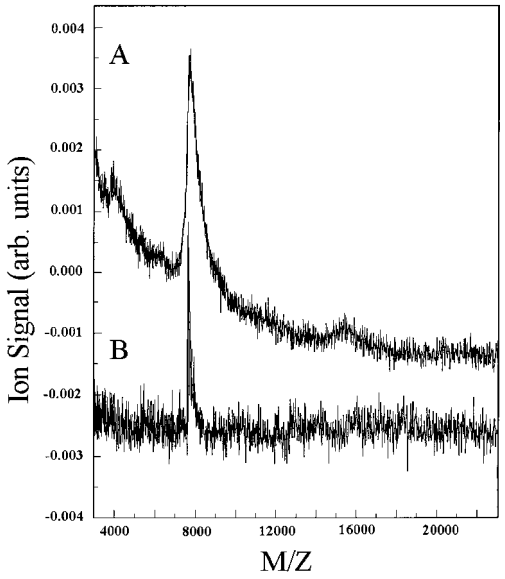

Figure 1. 25-Mer oligonucleotide mass spectra acquired (A) using laser at 355-nm wavelength for desorption and (B) using 1.064-μm laser for desorption.

The laser excitation at 355 nm demonstrated robust oligonucleotide detection from all samples prepared using 3-HPA as the matrix. However, when 532-nm or 1.064-μm radiation was used for desorption, no oligonucleotide peak was observed from samples when the thickness of the matrix layer was more than 10 μm.

Next, we changed the sample preparation procedure such that the same amount of DNA/matrix material could be deposited on a larger area of the substrate surface, thus effectively reducing the thickness of the matrix layer on the substrate. This was achieved by applying less concentrated DNA/matrix solution and depositing more solution on the surface to keep the same amount of analyte and matrix material. Figure 2 shows a stainless steel substrate with crystalized sample (A) prepared using 5 μL of DNA/3-HPA solution and (B) prepared by diluting the solution by a factor of 10 and depositing 50 μL of the stock on the substrate surface. The central area in Figure 2B is covered by a layer of DNA/matrix crystals less than 20-μm thickness from optical microscope examination. The central area of the "thin-layer" sample was used to acquire nonresonance MALDI mass spectra. Figure 1B shows an NR-MALDI mass spectrum of 25-mer oligonucleotide monitored at 1.064-μm laser excitation. The spectrum has been averaged over 16 laser shots. The laser fluence on the sample surface was 50 mJ/cm^2. The peak corresponding to the 25-mer appeared at m/z = 7620 Da. The resolution of 400 in Figure 1B (see also Figure 4B) is a factor of 20 better than the resolution of the UV-MALDI mass spectrum shown in Figure 1A. Figure 3 shows mass spectra of positive ions monitored from three consecutive laser shots to present shot-to-shot reproducibility of

(10) Tang, K.; Allman, S. L.; and Chen, C. H. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 943.
(11) Tang, K.; Taranenko, N. I.; Allman, S. L.; Chen, C. H.; Chang, L. Y.; Jacobson, K. B. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 673.

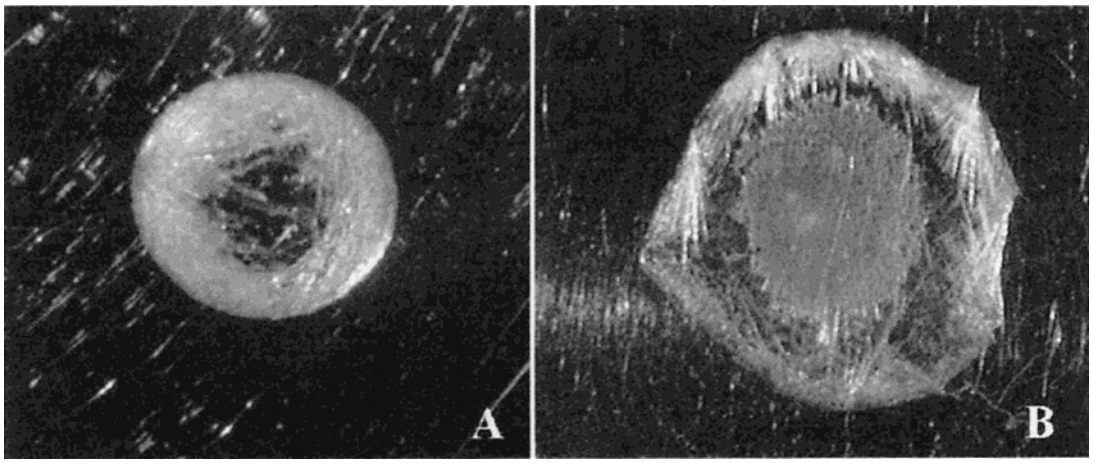

Figure 2. MALDI samples prepared (A) for UV-MALDI and (B) for NR-MALDI.

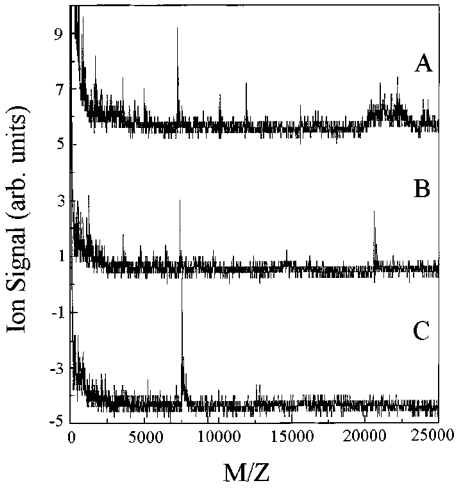

Figure 3. NR-MALDI mass spectra from three consecutive laser shots which are shown in (A−C), respectively. Mass spectra acquired from the central area of the sample shown in Figure 2B.

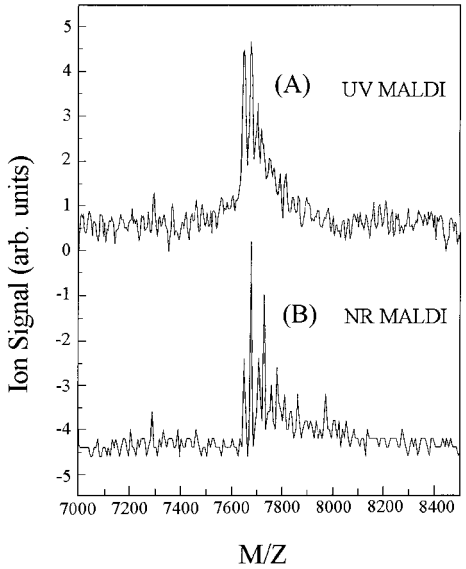

Figure 4. (A) UV-MALDI mass spectrum of 25-mer oligonucleotide acquired by mass spectrometry with delayed pulse extraction for improving resolution; (B) NR-MALDI mass spectrum acquired by mass spectrometry without using delayed pulse extraction.

NR-MALDI. Typically, the oligonucleotide signal was highest for the first laser shot and then gradually receded and disappeared within the next 5−15 laser shots. Some other peaks appeared in the spectra in Figure 3 and represent ions randomly scattered over a broad mass region. These peaks were not reproducible as can be concluded from the average spectrum in Figure 1B; therefore, the peaks were not studied systematically in this work. The mass spectrum detected under NR-MALDI conditions has some unique features as compared with UV-MALDI. Particularly, we found that the increase of laser energy above 50 mJ/cm^2 did not increase the amplitude of the nucleotide peak. It only caused the growth of the number of scattered ions over a broad mass region and made the detection of the nucleotide more difficult. Reducing the laser energy below 50 mJ/cm^2 decreased the amplitude of the nucleotide peak but did not significantly improve the reproducibility of the mass spectra. In these experiments, we found that the substrate plays an important role in the desorption of the oligonucleotide by NR-MALDI. The mass spectra presented in Figures 1, 3, and 4 were obtained when a stainless steel plate was used as the substrate. No oligonucleotide was observed with glass and quartz substrates, although an oligonucleotide was reliably detected from all type of substrates with UV-MALDI.

Figure 4 shows the comparison of mass spectra of the 25-mer nucleotide acquired under regular MALDI at 355 nm (A) and under NR-MALDI at 1.064 μm wavelength (B). The structure of the nucleotide peaks in Figure 4A and B is almost identical, and because the mass difference for the neighboring peaks is ∼23 Da, the peak structure can be assigned to sodium adducts. Although the resolution for both mass spectra is about the same, the high resolution in the spectrum in Figure 4A was achieved by using delayed pulse extraction, while comparable high resolution under NR-MALDI was obtained without using delayed pulse extraction. From comparison of the data in Figure 1 and Figure 4 we can conclude that the main gain in resolution both for NR-MALDI and for delayed pulse extraction under UV MALDI was achieved because of reducing energy spread for analyte ions.

Improvement of mass resolution also was observed in NR-MALDI spectra of pUC 18 samples as shown in Figure 5. The enzyme-digested sample contained fragments of 11, 18, 80, 102, 174, and 257 base pairs according to the information provided by the vendor (Sigma) and was confirmed independently by UV-MALDI spectrum shown in Figure 5A. The 20-fold increased

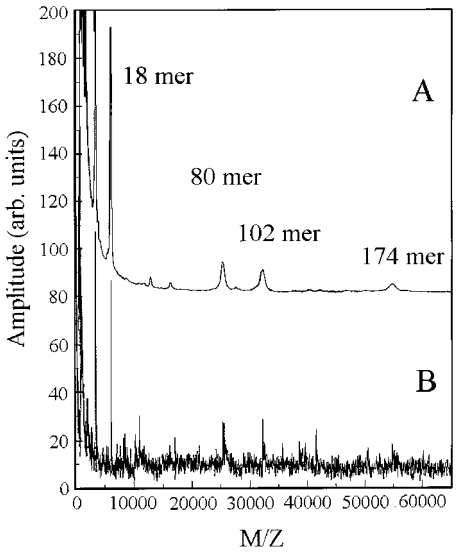

Figure 5. (A) mass spectrum of pUC18 sample acquired at 355-nm excitation using MALDI mass spectrometry with delayed pulse extraction; (B) NR-MALDI mass spectrum of the pUC 18 sample acquired without delayed pulse extraction.

spectral resolution observed for the 11- and 18-base pair fragments (see Figure 5B) decreased substantially, although still being 3−4 times better for fragments with masses greater than 100 base pairs. We believe the resolution of NR-MALDI spectra for large oligonucleotide fragments is limited mainly by the adducts attached to the analyte ions. It is very likely the number of adducts attached to each analyte is increasing with the size of the analyte molecule, and it can become the dominating factor of poor resolution for large DNA fragments.

Results similar to those shown in Figures 1 and 5 were obtained by using 532-nm laser beam for ablation. The only difference was that for mass spectra monitored at 532 nm the background noise from ions scattered over the broad mass range was stronger than that for 1.064 μm.

DISCUSSION

Some experimental facts observed for NR-MALDI of oligonucleotides can lead to conclusions about the underlying mechanisms of ion formation and desorption. First, we did not detect oligonucleotide signal under 1.064-μm and 532-nm excitation when the analyte was directly deposited on the substrate surface without matrix. The need for matrix for the NR-MALDI indicates that the matrix molecules might be involved in the formation of analyte ions through proton transfer or some kind of chemical ionization. Any photoinduced ionization of matrix and analyte molecules seems less likely since nonresonant laser excitation used for NR-MALDI does not efficiently produce this type of excitation.

Second, we did not succeed in detecting oligonucleotides with NR-MALDI from glass and quartz substrates. This might indicate that the matrix itself does not contribute directly to the desorption of analyte under NR-MALDI. The nucleotide signal was not detected when a thick layer of matrix has "isolated" substrate from the sample surface. The nucleotide signal was detected only when the sample preparation procedure was modified to reduce the thickness of the matrix layer. We believe desorption of analyte ions can be caused by an acoustic wave generated by a laser beam in the substrate. This assumption is supported by the fact that no signal was detected when substrates of transparent material (glass and quartz) were used. In that case, there was no significant energy release in the substrate, and as one can expect, no significant acoustic radiation came from the substrate into the matrix. The fact that oligonucleotide ions were not observed from samples when the thickness of the matrix layer was 10 μm or more could indicate that the bulk polycrystalline material can efficiently scatter the laser-induced acoustic radiation. Thereby it can reduce the intensity of acoustic radiation on the sample surface. This can explain the need for thin-layer samples with NR-MALDI. An acoustic mechanism of desorption can also explain the improvement of mass resolution in the NR-MALDI spectra. If matrix evaporation is not required for desorption, there are no collisions of fast matrix molecules and analyte ions that could cause significant spread in the kinetic energy of the ions.

Third, the disappearance of the nucleotide signal from one spot on the sample surface after a few laser shots indicates that the analyte ions came from the surface, not from the bulk of the sample. The ion signal disappears as soon as all of the analyte molecules are expelled from the surface by the desorption process. Similar effects have been observed recently for desorption of electrons and small ions from solid surfaces induced by short-duration acoustic pulses.[8,9]

The desorption of analyte ions from the surface can also be used to explain the lower sensitivity of the NR-MALDI signal as compared with UV-MALDI signal from the same sample. We believe that only a small fraction of nucleotide molecules are distributed near the surface and can be desorbed by acoustic waves as compared with the total number of molecules loaded into the sample and available for UV-MALDI detection.

It should be pointed out here, that the use of thin-layer samples might also be favorable for thermal desorption from an overheated substrate surface.[12,13] In fact, the poorly reproducible peaks that were observed in broad mass range can be attributed to thermal desorption of atoms and residual gas molecules from the substrate surface. However, it is still doubtful that the thermal process can desorb large molecules without significant decomposition. It is very unlikely the thermal desorption can provide high-resolution spectra similar to that presented in Figures 4B and 5B, since the thermal process is usually accompanied by slow cooling, which adds long tails to the peaks, thus reducing resolution.[12,13]

ACKNOWLEDGMENT

This research was sponsored in part by the National Institute of Justice and in part by the Office of Health and Biological Research, Natural and Accelerated Bioremediation Research, U.S. Department of Energy under contract DE-AC05-00OR22725 with UT-Battelle, LLC. Preparation of the manuscript by Darlene Holt is acknowledged.

Received for review August 22, 2000. Accepted November 28, 2000.

AC001006+

(12) Beuhler, R. J.; Friedman, L. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 1.

(13) Handschuh, M.; Nettesheim, S.; Zenobi, R. *J. Phys. Chem.* **1999**, *103*, 1719.