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COMPARISON OF DESALTING METHODS FOR MALDI-TOF MS ANALYSIS OF OLIGONUCLEOTIDES

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Introduction

Nucleic acids are utilized in many areas of molecular biology including DNA sequencing, polymerase chain reaction (PCR), diagnostics, and antisense therapeutics. Therefore, they require some measure of quality control (QC). Due to the large volume of oligonucleotides that are currently synthesized, rapid methods are needed for analysis. Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-ToF MS) has proven to be a very rapid method for qualitative analysis of oligonucleotides. Analysis times have been shown to be on the order of a few seconds per sample, which is suitable for very high throughput applications (> 20,000 samples per day).

Sample preparation for MALDI-ToF MS is the key for successful analysis of nucleic acids. The samples are often contaminated with alkali cations, detergents, and other constituents from polymerase chain reaction buffers, undermining the quality of mass spectra (Fig. 1). However, sample preparation prior to MALDI MS requires significant time to perform, and presents the bottleneck for high throughput analysis.

A modified MALDI target plate was previously designed for on-target cleanup of peptides (Fig. 2). The derivatized plate surface allowed for direct desalting of protein digests on-target prior to MALDI, significantly decreasing sample preparation time. This technology was also applied for oligonucleotides; however, with less encouraging results. Therefore, several methods for fast and robust nucleic acid sample cleanup prior to MALDI MS analysis were evaluated (Fig. 3). These included solid phase extraction (SPE), incubation with ion-exchange (IEX) beads, ZipTips®, NuTips™, and MALDI targets that were chemically modified with reversed-phase (RP) coatings. Comparisons of these methods to each other and to liquid chromatography electrospray ionization-mass spectrometry (LC ESI-MS) were performed (Fig. 4).

Experimental

MALDI MS: A Waters® Micromass® MALDI LR™ was used for all experiments. The instrument was operated in the positive linear mode. Pulse 1200 V; source 15000 V; MCP 1850 V; matrix suppression delay = 500 amu. The instrument was operated by Waters® MassLynx™ Software, version 3.5

MALDI Matrix: 25 mg of 3-hydroxypicolinic acid (3-HPA) was rinsed with 100 µL of 100% acetone and subsequently dissolved in 1 mL of 25% acetonitrile in dH_2O.

Desalting: Waters MassPREP™ PROtarget™ sample preparation plates, Oasis® HLB 3 cc cartridges (60 mg, 30 µm sorbent), and Oasis® HLB µElution plates. NuTips™ were provided by Glygen (Columbia, MD, USA) and ZipTips® μC_{18} were provided by Millipore (Bedford, MA, USA). Modified MALDI targets were prepared in-house on regular stainless steel plates. Various hydrophobic sorbents were used to modify MALDI target surfaces. Details of desalting protocols for each device can be found in Table 1.

Samples: Oligonucleotides were purchased from the vendor and used without further purification. See figure details for sample concentrations and mass loads on-target.

Results and Discussion

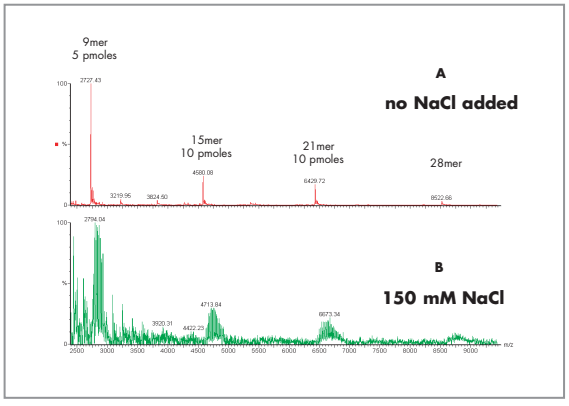

Figure 1: Sample preparation (desalting) is critical for successful MALDI MS analysis of nucleic acids (Fig. 1A). The presence of alkali cations in the oligonucleotide solution severely decreases sensitivity and complicates spectrum interpretation (Fig. 1B).

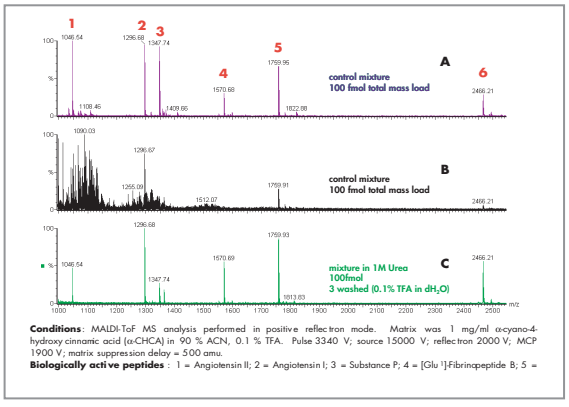

Figure 2: Impact of on-target sample cleanup of a mixture of 6 biologically active peptides. Signal suppression is evident when the sample is analyzed in 1M urea (Fig. 2B). The chemically modified MALDI plate (MassPREP PROtarget) allows rapid sample preparation on-target, and dramatically enhances signal sensitivity and decreases spectrum complexity (Fig. 2C). This method was found to be inadequate for oligonucleotides.

Desalting Device / Desalting Step	Millipore ZipTip® µC18	Glygen NuTip™ C18	MassPREP™ PROtarget™	Oasis® HLB µElution plate (96-well format)
Condition	50% ACN[1] in dH2O	50% ACN in dH2O	50% ACN in dH2O	50% ACN in dH2O
Equilibration	0.1M TEAA[2], pH 7	0.1M TEAA, pH 7 or 5 mM DMBAA, pH 7	12.5 mM TBAH[3]	5 mM DMBAA[4], pH 7
Load	0.1M TEAA, pH 7	0.1M TEAA, pH 7 or 5 mM DMBAA, pH 7	150 mM NaCl	5 mM DMBAA, pH 7
Wash 1	0.1M TEAA, pH 7	0.1M TEAA, pH 7 or 5 mM DMBAA, pH 7	dH2O	5 mM DMBAA, pH 7
Wash 2	dH2O	dH2O	dH2O	5 mM DMBAA, pH 7
Elution/Desorption	25 mg/ml 3-HPA[5] in 25 % ACN in dH2O	25 mg/ml 3-HPA in 25 % ACN in dH2O	25 mg/ml 3-HPA in 25 % ACN in dH2O	25 % ACN in dH2O

Abbreviations: [1]ACN = acetonitrile; [2]TEAA = triethylammonium acetate; [3]TBAH = tetrabutylammonium hydroxide; [4]DMBAA = dimethylbutylammonium acetate; [5]3-HPA = 3-hydroxypicolinic acid.

Table 1: Reagents and procedures for various desalting devices utilized for nucleic acid sample preparation prior to MALDI-ToF MS analysis. Several different methods of oligonucleotide desalting were evaluated and compared. These included SPE devices in the 96-well and pipette tip format, as well as chemically modified MALDI surfaces for on-probe sample preparation.

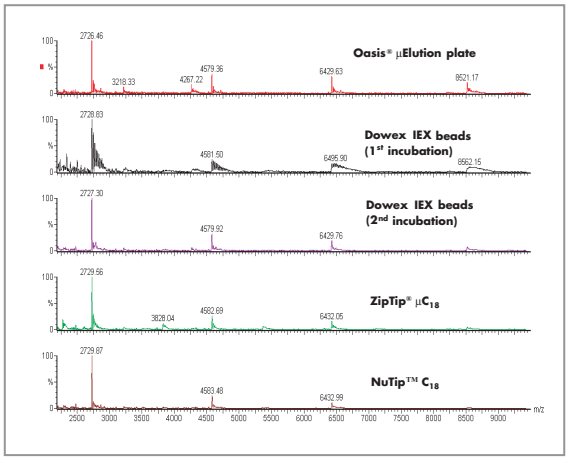

Figure 3: MALDI-ToF MS analysis of an oligonucleotide mixture desalted by various methods. Original sample was contaminated with 150 mM NaCl. SPE devices (Oasis® HLB µElution plates) and Dowex ion-exchange particles (2 incubations) are adequate for alkali cation removal from nucleic acids. This is also the case for ZipTip® µC18 and NuTips™. The chemically modified MALDI surface (MassPREP PROtarget) is less efficient. It is probable that the oligonucleotides do not bind during the load step, and are washed off the target surface prior to desorption.

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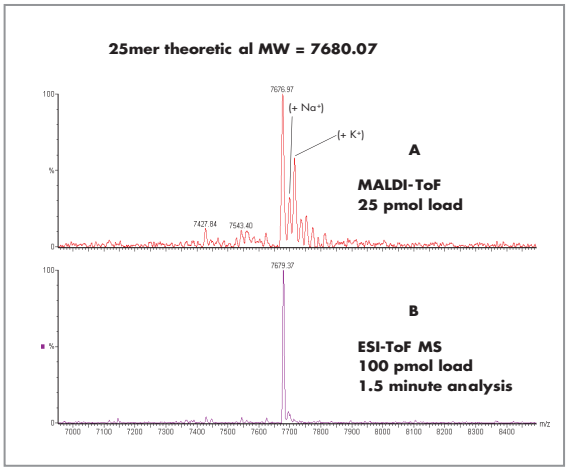

Figure 4: Comparison of MALDI (A) and ESI-MS (B) for analysis of a 25-mer synthetic heterooligonucleotide. The sample was analyzed by MALDI-ToF MS after being desalted by µSPE (Fig. 4A). Duty cycle times for this method were under 1 minute per sample (data acquisition time only). Some alkali adduction (Na^+, K^+) was still noticeable. Alternatively, high performance LC was utilized on-line as a desalting technique prior to ESI-MS (Fig. 4B). Oligonucleotide samples were consistently well desalted, and minimal cation adduction was observed. Cycle times for this method were 1.5 minutes per sample.

Conclusions

- Sample desalting is the critical parameter for successful analysis of nucleic acids by MALDI-ToF MS.
- Desalting of nucleic acids off-line prior to MALDI is time-consuming and labor-intensive. On-probe desalting using modified MALDI surfaces is a simpler approach.
- The MassPREP PROtarget showed limited success for on-target nucleic acid desalting.
- Desalting of contaminated samples by ZipTip™ μC_{18}, SPE, or incubation with Dowex beads are alternative techniques. Some sample losses were observed for ZipTip™ μC_{18} and NuTips™.
- Nucleic acid preparation prior to MALDI by µSPE (Oasis® HLB) is efficient, and is also capable of sample concentration.

Oligonucleotide desalting by on-line HPLC prior to ESI-MS is complementary to off-line methods utilized before MALDI-ToF MS analysis.

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