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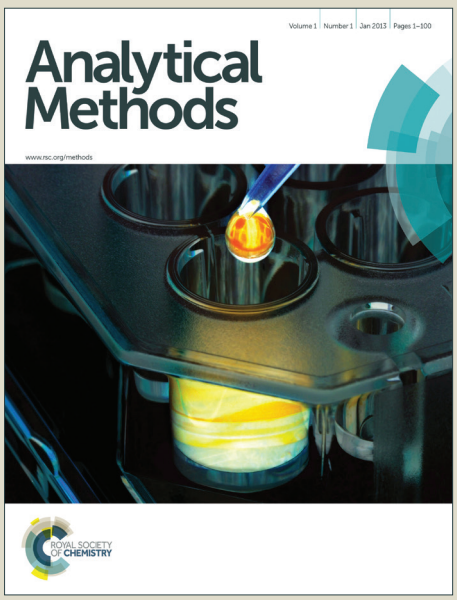

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ARTICLE

Nanomaterials Based Mass Spectrometry of Oligodeoxynucleotide-Drug Complexes

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A surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) using HgTe nanostructures as the matrix has been employed for the detection of four 15-base oligodeoxynucleotides (ODNs) that are genes found in acute myeloid leukemia (AML) patients and their complexes with mitoxantrone (MTX) that is a common drug for treatment of AML patients. The major peaks for the four tested ODNs are at *m/z* values of 4571, 4586, 4610, and 4635, while they are at *m/z* values of 5017, 5031, 5055, and 5079 for their corresponding complexes with MTX. The ODN with *m/z* value of 4610 is assigned for a normal gene of AML, while the other three are single-base mutant ODNs. This approach allows detection of the tested ODNs at the concentrations down to 2 nM, showing their potential for diagnosis of AML. The dissociation constants values of the four tested ODN-MTX complexes determined by the SALDI-MS approach are similar and all in the μM level, which agree with that determined by applying a conventional absorption approach. Relative to the conventional approach, the SALDI-MS approach has advantages of simplicity, rapidity, reproducibility, and use of smaller amounts of ODNs and MTX.

Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a useful tool for rapid identification of large biomolecules, including proteins, peptides, and nucleic acids.[1, 2] Large shot-to-shot and sample-to-sample variations are common in MALDI-MS, mainly because of inhomogeneous co-crystallization of analytes with traditional organic matrices and chemical complexity.[3, 4] In addition, it is uneasy to detect oligodeoxynucleotides (ODNs) greater than 25 bases through MALDI-MS, mainly due to difficult ionization of larger ODNs when using common organic matrices such as 3-hydroxypicolinic acid (HPA) and 2,5-dihydroxybenzoic acid (DHB).[5 - 7] Since MALDI-MS is usually conducted under acidic conditions in order to have efficient proton transfer from the matrix to ODNs, it is difficult to investigate the interactions of ODNs with interesting targets such as drugs and proteins.[8, 9] Although electrospray ionization mass spectrometry (ESI-MS) has been applied to investigate ODN–small molecule complexes,[10-13] it suffers from poor sensitivity and a lower *m/z* limit. Detection of ODNs with amounts less than 100 pmole and/or with sequences larger than 22 base pairs is difficult through ESI-MS.[14-16] In addition, the MS data obtained may not correspond correctly to complexes in liquid solution as a result of different stabilities of the complexes in the liquid and gas phases; during ESI-MS analysis electrostatic attraction is usually strengthened, while hydrophobic interaction is weakened or unaffected.[17, 18] In addition, ODN-drug complexes are usually stable in the presence of salt such as 100 mM NaCl, salt induced suppression of analyte MS signals however occurs, leading to reduced sensitivity.[19, 20]

MALDI-MS techniques using nanomaterials over organic matrices have demonstrated for the determination of small molecules, proteins, and DNA, with advantages of better reproducibility and less MS signal background in the low *m/z* region.[21-24] The nanomaterials based MALDI-MS technique is often called surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS). In addition, nanomaterials can be used as selective elements to concentrate analytes of interest and as the matrix like a traditional organic matrix to absorb laser energy to induce desorption and ionization of the concentrated analytes.[5, 25]

SALDI-MS using HgTe nanomaterials can be conducted under mild conditions, allowing investigation of protein-protein and protein-drug interactions.[5] HgTe nanostructures with low thermal conductivity and low melting point allow generation of high laser-induced temperatures for the detection of biopolymers such as proteins and DNA.[8, 26-29] For example, SALDI-MS using HgTe nanostructures allowed detection of ss-ODNs (up to 50-mer) and ds-ODNs (up to 30 base pairs), with LODs down to femtomoles and sample-to-sample variations less than 23%.[9] The study suggested that SALDI-MS using HgTe nanostructures is potential for screening of drugs that have affinities for particular DNA targets, with rapidity, simplicity, and sensitivity, which shall be beneficial for the pharmaceutical industry.[30]

In this study, a similar SALDI-MS approach using HgTe nanostructures was applied for rapid and sensitive detection of weak ODN-drug complexes. The MS data allowed for determination of their dissociation constants (K_d) values simultaneously. Four ODNs, including one normal gene and

three mutants found in acute myeloid leukemia (AML) patients, and a drug mitoxantrone (MTX) that is commonly used to treat the AML patients were tested in this study.[31] Important factors such as pH (4.0–9.0) and concentrations of ammonium citrate (10–200 mM), cadaverine (0.05–30 μM), and Ag^+ (0.1–10 μM) were investigated to optimize the MS signals. Relative to common absorption and fluorescence approaches,[32] the SALDI-MS provided advantages of high throughput and use of less amounts of samples for the determination of the formation complexes of ODN-drug complexes.

Experimental

Materials

Cadaverine, citric acid, mercaptopropionic acid (MPA), MTX, silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), and sodium hydroxide (NaOH) were purchased from Aldrich (Milwaukee, WI). Ammonium hydroxide (NH_4OH), ethanol (EtOH), mercury chloride ($HgCl_2$), and tellurium powder were obtained from Acros (Geel, Belgium). The sequences of the four ss-ODNs used in this study are listed in Table 1. For simplicity, the four ODNs are separately presented as WT, M1, M2 and M3. HgTe nanostructures (network structures) were prepared from hydrogen telluride (NaHTe) solution and $HgCl_2$ in the presence of MPA under alkaline condition according to the literature.[8] Tellurium powder (0.127 g) and $NaBH_4$ solution (2 M, 1 mL) were mixed to prepare NaHTe solution. For simplicity, the concentration of the purified HgTe nanostructures is denoted as 1X.

Table 1 Sequences and adduct ions of ss-ODNs used in this study

ODNs	Sequence (5'→3')	Size (mer)	$[M + H]^+$ (m/z)
WT	5'-GCT CGA **GAT** ATC ATG-3	15	4610
M1	5'-GCT CGA **T**AT ATC ATG-3'	15	4586
M2	5'-GCT CGA GA**G** ATC ATG-3'	15	4635
M3	5'-GCT CGA **C**AT ATC ATG-3'	15	4571

Analysis of ODNs and ODN-MTX complexes

To optimize the experimental condition for SALDI-MS analysis of ODNs and their complexes with MTX (1 μM), ammonium citrate solutions (10–200 mM) over a pH range (4.0–9.0), cadaverine (0.05–30 μM), Ag^+ (0.1–10 μM) and HgTe nanostructures (1–8 X) were evaluated. Herein, only experimental processes under the optimal condition are provided. The tested ODNs were diluted with ultrapure water to prepare corresponding stock solutions, each with a concentration of 100 μM. The stock ODN solutions were mixed and then diluted with ultrapure water to 100 μL, each with final concentration ranging from 0.01–10 μM. For the preparation of ODNs-drug mixtures, the four ODN stock solutions (each 5 μL, 10 μM) were added to each of the solutions that had been prepared by mixing water (140 μL) with ammonium citrate solutions (20 μL, pH 7.0, 500 mM) and MTX (20 μL, 0.01–2 mM). The mixtures (200 μL) were equilibrated at ambient temperature (25 ℃) for 1 h. Aliquots (200 μL) of the mixtures were mixed with HgTe nanostructures (2X, 50 μL) under gentle shaking at ambient temperature for 30 min. After conducting two cycles of centrifugation (16099 g, 10 min)/wash (ultrapure water, 250 μL) to remove extra salts, the pellets were then dispersed separately in aliquots of mixtures (50 μL) of 1 μM Ag^+, 10 μM cadaverine, and 30 mM ammonium citrate (pH 7.0). Aliquots (2.0 μL) of the mixtures were pipetted onto a stainless-steel 96-well MALDI target (Bruker Daltonics, Bremen, Germany) and dried in air at ambient temperature for 30 min prior to SALDI-MS analysis.

SALDI-MS

A Microflex MALDI-TOF mass spectrometer (Bruker Daltonics) was operated in a linear positive-ion mode at an applied acceleration voltage of 20 kV for all MS experiments. The samples were irradiated with a nitrogen laser (output at 337 nm) at 10 Hz. The laser energy was set slightly higher than the threshold. As-generated ions were stabilized energetically during a delayed extraction period of 200 ns and then accelerated through the time of flight in the reflection mode before entering the mass analyzer. Each mass spectrum was generated by averaging over 300 laser pulses, allowing good resolution and high signal-to-noise (S/N) ratios.

UV-visible absorption

Aliquots of MTX solution (100 μL, 100 μM) were mixed separately with ODNs solutions (900 μL, 0.56–11.1 μM) that had been prepared in ammonium citrate solution (pH 7.0, 55.6 mM). The mixtures were equilibrated at ambient temperature for 1 h. A double-beam UV−Vis spectrophotometer (Cintra 10e, GBC, Dandenong, VIC, Australia) was used to record the absorption spectra of the ODN-MTX complex solutions. All spectra were acquired in the wavelength range of 500-800 nm.

Results and discussion

The optimal conditions were found to be pH 7.0, 30 mM ammonium citrate, 10 μM cadaverine, and 1 μM Ag^+, with respects to S/N ratios of ODNs.[9] Ammonium ions as proton donors assisted the ionization of the ODNs. Cadaverine has high proton affinity (pKa = 9.13 and 10.25) with ODNs and the protonated cadaverince cations further acted as proton sinks for ODNs during the ionization process.[33] Thus, addition of cadaverine prevented the protonation of ODNs in early stage. On the other hand, Ag^+ was useful to stabilize ODNs during ionization process through forming strong C-Ag-C complexes.[34] As a result, higher MS signals with narrower peak profiles were obtained in the presence of cadaverine and Ag^+. Under such conditions, ss-ODNs were detected at concentrations down to the femtomole level. Upon increasing the concentration of HgTe nanostructures from 2X to 8X, the signal to noise ratio of $[ODN]^+$ adducts decreased, mainly because of decreased surface density of ODNs on HgTe nanostructures and increased MS background signals. Upon decreasing the concentration of HgTe nanostructures from 2X to 1X, the signal decreased as a result of inefficient absorption of energy from laser light, leading to lower S/N ratios of the ODNs. Figure 1 shows the mass spectrum of a representative

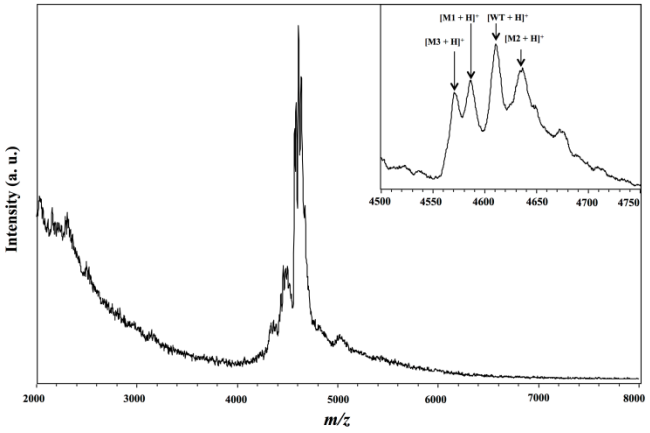

Figure 1 SALDI mass spectrum of a mixture of ODNs using HgTe nanostructures as the matrix. The sample was prepared in a solution (pH 7.0) containing 30 mM ammonium citrate, 1 μM Ag+, and 10 μM cadaverine. Inset: mass spectrum showing the peaks detected over an m/z range from 4500 to 4750. Intensity is plotted in an arbitrary unit (a. u.).

ODNs mixture (each at 0.25 μM) through SALDI-MS under the optimal conditions. The major peaks are at m/z values of 4571, 4586, 4610, and 4635, which are assigned for the $[M3 + H]^+$, $[M1 + H]^+$, $[WT + H]^+$, and $[M2 + H]^+$, respectively. Table 2 lists the linear ranges, linear regression coefficients, and limits of detection (LOD) at an S/N ratio of 3 of the ODNs in the mixtures, each with LOD of 2 nM. The LOD in term of absolute amount for each ODN was 16 femtomole, which is lower than that (2 picomole) obtained by ESI-MS.[16] Batch-to-batch variations in terms of MS signals for the ODNs were smaller than 29% (n = 5). Because the four tested ODNs are genes found in AML patients, the result shows that the SALDI-MS approach is sensitive for the diagnosis of AML. Figure 2 shows the mass spectra ODNs and ODN-MTX complexes, with peaks at *m/z* values of 5017, 5031, 5055, and 5079 that are assigned for the $[M3 + MTX + H]^+$, $[M1 + MTX + H]^+$, $[WT + MTX + H]^+$, and $[M2 + MTX + H]^+$ adducts, respectively. The result revealed that the ODNs and MTX formed complexes in a fashion of 1:1. To the best of our knowledge, this is the first example of SALDI-MS for the detection of ODN-drug complexes. The MS signals were stable at the laser power from 141 to 149 μJ. Further increases in the laser power lead to higher background signals. The sensitivity for the ODNs and ODN-MTX complexes becomes poor when the laser power is less than 132 μJ, mainly because of their poor desorption/ionization efficiency. The SALDI-MS allowed differentiation of MTX-ODN complexes with only one base mutation among the four ODNs. The planar anthraquinone ring of MTX intercalated with the ODN between its base pairs, while its nitrogen-containing side chain electrostatically interacted with the negatively charged phosphate backbone of the ODNs, which was evident with a hypochromic effect and

Table 2 Linear ranges (LR), regression coefficients (R^2), and LODs (S/N = 3) of detection of a mixture of ODNs provided by SALDI-MS using HgTe nanostructures.

ODNs	LR (μM)	R^2	LOD (μM)	$[M + H]^+$ (m/z)
WT	0.0－0.5	0.999	0.002	4610
M1	0.01－1	0.998	0.002	4586
M2	0.01－1	0.984	0.002	4635
M3	0.01－1	0.977	0.002	4571

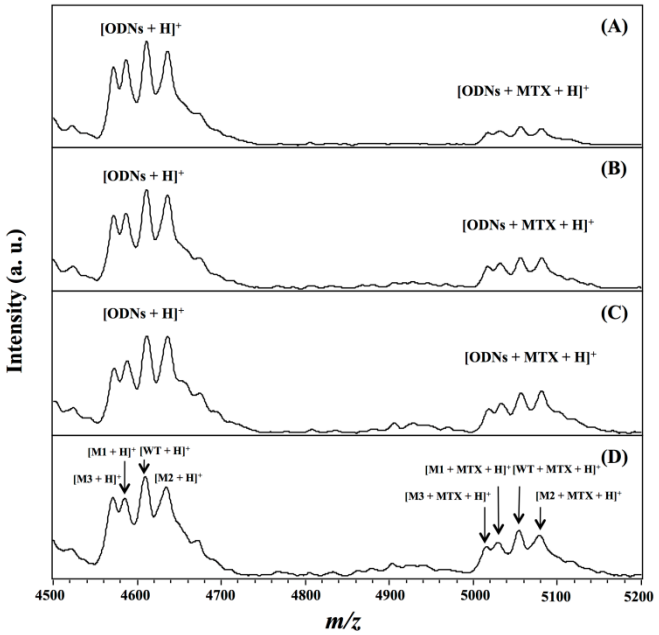

Figure 2 Representative SALDI mass spectra of ODNs (each at 0.25 μM) in the presence of various concentrations of MTX using HgTe nanostructures as the matrix. MTX concentrations: (A) 10, (B) 30, (C) 50, and (D) 100 μM. The conditions were the same as those described in Figure 1.

bathochromic shift in their absorption.[35]

The MS spectra (Fig. 2) obtained at a constant concentration of ODNs (1 μM) and various concentrations of MTX (1–200 μM) were used to determine the K_d values of MTX complexes with the four ODNs. The saturated concentration was estimated to be about 50 μM. By plotting the MS signal ratio of ODN- MTX complexes/ODNs against [MTX] as shown in Fig.3, their K_d values were determined according to Eqn. 1:

$$K_d = [\text{ODNs}]\,[\text{MTX}]/[\text{ODNs-MTX}] \quad (1)$$

The K_d values were determined to be 15.6 ± 3.1, 16.1 ± 3.6, 15.9 ± 3.4 and 14.3 ± 2.2 μM for WT-MTX, M1-MTX, M2-MTX, and M3-MTX complexes, respectively. To confirm the obtained formation constants, absorption spectra of MTX in the

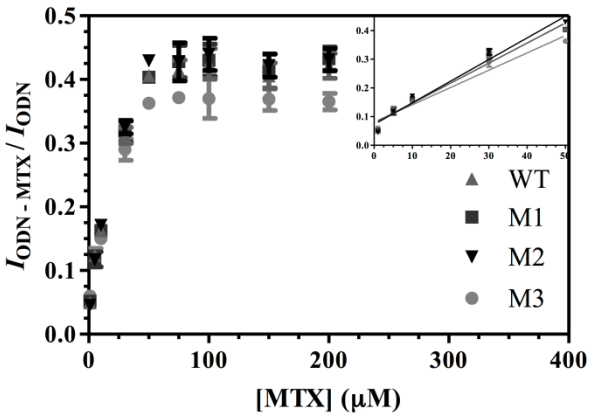

Figure 3 Plots of relative MS signal ratios ($I_{ODN-MTX}/I_{ODN}$) of ODNs over ODN-MTX complexes against [MTX]. The experimental conditions were the same as shown in Fig. 2. Insets: concentrations of MTX were from 1 to 50 μM.

presence of one of the four ODNs at various concentrations were recorded. Absorption spectra were used separately to calculate the formation constants (K) of MTX-ODN complexes according to Eqn. 2:

$$A_0/[A - A_0] = \varepsilon_G/[\varepsilon_{H\text{-}G} - \varepsilon_G] + \varepsilon_G/K\,[ODN][\varepsilon_{H\text{-}G} - \varepsilon_G] \quad (2)$$

where A_0 and A are the absorbance values at 610 nm of the drug in the absence and presence of ODNs, ε_G and ε_{H-G} are the absorption coefficients of the drug and its complex with ODNs at 610 nm, respectively.[36] Representative absorption spectra of MTX-WT complexes at three different concentrations of WT are displayed in Fig. 4A. From the plots of $A_0/(A - A_0)$ versus 1/[ODN] (Figure 4B-E), the K values for WT-MTX, M1-MTX, M2-MTX, and M3-MTX were determined to be 7.6×10^4, 1.3×10^5, 1.4×10^5, $8.6 \times 10^4\,M^{-1}$ (K_d were 13.1 ± 10.3, 7.8 ± 2.9, 6.9 ± 3.8 and 11.6 ± 4.1 μM), respectively. The K_d values obtained from the SALDI-MS measurement agree with that calculated form the absorption data, mainly because the ODNs interacted with MTX through electrostatic interactions, co-crystallization processes was avoided, and the SALDI-MS was operated under a mild condition.[11] We point out that the SALDI-MS is much more sensitive (2 nM vs. 0.5 μM ODNs) than that of the absorption approach. The total times required for obtaining the four K_d values by conducting absorption and SALDI-MS measurements were about 4 and 2 h, respectively. For the absorption and MS studies, greater amounts of MTX (240 vs. 58 nmole) and ODNs (9.25 vs. 0.45 nmole) were used for the absorption measurement relative to the SALDI-MS measurement. The SALDI-MS approach combined with HgTe nanostructures allowed a sensitive detection of ODN-MTX complexes with the advantages of high throughput, reproducibility, and less amount of sample required.

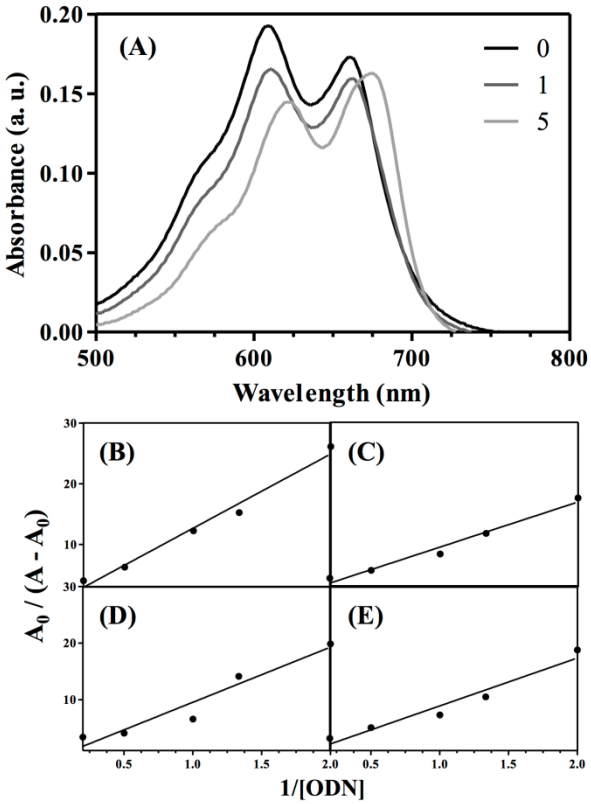

Figure 4 (A) Representative absorption spectra of WT-MTX complex solutions at various concentrations of MTX and (B)–(E) plots of $A_0/(A - A_0)$ versus 1/ [ODN] for WT, M1, M2, and M3, respectively. (A): the concentrations of WT were (a) 0, (b) 1, and (c) 5 μM. The concentration of MTX was kept at a constant of 10 μM, while those for ODNs were in the range of 0.5–5 μM in (B)-(E). Absorbance is plotted in arbitrary units (a. u.).

Conclusions

A simple and sensitive SALDI-MS approach using HgTe nanostructures was employed for the detection of ODNs and weak ODN-drug complexes. This approach provided a LOD of 2 nM for the tested ODNs, showing that it holds great potential for the diagnosis of AML. Relative to conventional optical approaches, the present SALDI-MS approach allows high-throughput determination of the K_d of MTX-ODN complexes. The present SALDI-MS approach is useful for differentiation of the complexes of MTX with ODNs having sequences with only a single-base difference, revealing its potential for screening of gene based drugs.

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Notes and references

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