Biochemical Assays of Immobilized Oligonucleotides with Mass Spectrometry

Haim Tsubery and Milan Mrksich*

Department of Chemistry and Howard Hughes Medical Institute, The University of Chicago, Chicago, Illinois 60637

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This paper reports the use of mass spectrometry to characterize oligonucleotides immobilized to the surfaces of biochips. Biotinylated oligonucleotides were immobilized to self-assembled monolayers that present a streptavidin layer and then treated with a complementary strand to present short duplexes. Treatment of the surface with 5-methoxysalicylic acid and ammonium citrate matrix allows the individual oligonucleotides to be observed by matrix-assisted laser desorption/iozation and time-of-flight mass spectrometry (MALDI-TOF MS). Examples are shown wherein this method is applied to assays of hybridization, of cleavage by a deoxyribozyme, of a dephosphorylation reaction, and of the adducts formed on treatment of DNA with *cis*-platin. This work provides an early example of the application of mass spectrometry to DNA biochips and may substantially expand the applications of the now common oligonucleotide arrays.

Introduction

Oligonucleotide arrays have transformed the analysis of gene expression and now provide a routine tool for the global mapping of expression patterns that characterize cellular states and activities.[1,2] These assays are based on the hybridization of fluorescently labeled DNA strands to oligonucleotides patterned into an array and the use of fluorescent scanning to quantitate the target DNA at each element of the array. The arrays combine the benefits of solid phase assays, which permit a simple separation of analytes from the reaction mixture, and the high-throughput performance of massively parallel assays. Yet, they have found only limited use in assays other than hybridization, including those of the reaction of DNA with toxins, drugs, and enzymes. This limitation stems from the difficulty in labeling and analyzing adducts of small molecules and DNA, including those formed on reaction of DNA with the cancer drug *cis*-platin[3] or the natural products neocarzinostatin[4] and bleomycin.[5,6] This paper reports an assay format that combines matrix-assisted laser desorption–ionization (MALDI) mass spectrometry and self-assembled monolayers presenting oligonucleotides for performing assays of DNA-directed activities. The SAMDI assay, self-assembled monolayer for matrix-assisted laser desorption–ionization mass spectrometry, is compatible with array formats and requires only that the mass of the reaction product is distinct from that of the immobilized probe, making the assay versatile and straightforward.

Several groups have pursued the goal of utilizing label-free methods for the analysis of oligonucleotide arrays. Buc and co-workers have used surface plasmon resonance (SPR) spectroscopy to measure the hybridization of DNA with immobilized probes and to quantitate the elongation of bound oligonucleotides by reverse transcriptase.[7] Corn and co-workers used SPR imaging to monitor the interaction of binding proteins to an array of DNA duplexes having either single-base mismatches or two-base insertions.[8] Okahata and co-workers used the quartz crystal microbalance (QCM), which is based on changes in the resonant frequency of a quartz crystal with accumulation of mass at its surface, to monitor Klenow fragment polymerase reactions, allowing distinct monitoring of the binding of polymerase to the immobilized primer, elongation of the complementary strand along the template, and release of the enzyme from the polymerized DNA.[9] Wang and co-workers used QCM to monitor the hydrolysis of dsDNA and ssRNA molecules by DNase I and DNase A, respectively.[10]

Mass spectrometry represents another approach to label-free detection of biological analytes and has the benefit of providing molecular information on the analyte. While SPR and related optical methods provide an aggregate signal that is related to the total amount (both the molecular weight and density) of all analytes at an interface, mass spectrometry identifies each species by its mass to charge ratio (*m*/*z*). It is therefore better-suited to discriminating adducts that have a minimal shift in mass, to analyzing mixtures, and to resolving intended analytes from background. Indeed, mass spectrometry methods have been important in analyzing DNA adducts that are present in solution,[11–14] but attempts to apply MALDI-TOF MS to the analysis of covalently immobilized oligonucleotides–including arrays of oligonucleotides–have proven unsuccessful.[15] Köster and co-workers reported that biotinylated oligonucleotides bound

* To whom correspondence should be addressed. E-mail: mmrksich@uchicago.edu; (fax) 773-702-1677.

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to magnetic or glass beads also could not be detected by MALDI-TOF MS.[16] Shchepinov and co-workers prepared oligonucleotides having an acid-labile P3′−N5′ phosphoramide bond that is cleaved in the presence of matrix, and which then permitted the observation of peaks corresponding to the released DNA fragment.[17] Rothschild and co-workers captured oligonucleotides on streptavidin-coated beads incorporating a photocleavable biotin linker and could then photochemically release and observe the DNA on irradiation by the near-UV source in the MALDI instrument.[18] The direct analysis of oligonucleotides present in array formats, however, has not been performed with mass spectrometry methods.

We have previously described the use of MALDI-TOF MS to characterize self-assembled monolayers of alkanethiolates on gold.[19] In this technique, termed SAMDI, a commercial instrument is used to irradiate the monolayer with a nitrogen laser, which results in desorption and ionization of the alkanethiolates–or the corresponding dialkyldisulfide molecules–from the gold substrate without significant fragmentation. The mass spectrum therefore reveals *m*/*z* peaks that correspond to the mass of the substituted alkanethiolates and provides data that is readily interpreted. We have demonstrated the use of peptide-modified monolayers to measure kinase,[20] protease,[21] and methyl transferase activities.[22] We have also applied SAMDI to binding assays of soluble[23] and membrane-bound proteins,[24] to high-throughput screens,[25] and to immunoassays of proteins in clinical samples.[26] These examples establish the generality of SAMDI in label-free assays of a broad range of biological activities. The aim of the current work is to extend the SAMDI method to assays of the reactions of oligonucleotides, and particularly to those assays that are currently difficult to perform using oligonucleotide arrays. We first show that immobilization of oligonucleotides by way of a streptavidin linker, but not through a direct covalent tether, permits observation of clear peaks for these molecules, and we then demonstrate assays of the cleavage of an oligonucleotide by a deoxyribozyme, of a dephosphorylation reaction by T4 polynucleotide kinase and of the adducts formed by treatment of an oligonucleotide with *cis*-platin.

Experimental Section

Materials. All oligonucleotides were purchased from Integrated DNA Technologies. 5-Methoxysalicylic acid, streptavidin, dithiothreitol, cystamine-2HCl, and *cis*-diamineplatinum (II) dichloride were purchased from Sigma. Ammonium acetate dibasic was obtained from Fisher chemicals. Phosphate-buffered saline (PBS) was purchased from Gibco. Glass microscope coverslips for gold depositions were obtained from Fisher Scientific. Biotin-*N*-hydroxysuccinimide ester was purchased from Pierce.

Synthesis of *N*-Biotinylcysteamine. *N*-Methylmorpholine (19.4 μL, 176 μmol) was added to a solution of biotin-*N*-hydroxysuccinimide ester (50 mg, 146 μmol) and cystamine-2HCl (20 mg, 88 μmol) in a mixture of DMF and H_2O (1.4 mL; 6:1), and the reaction mixture was stirred for 24 h at 25 °C. Dithiothreitol (46.2 mg, 300 μmol) was added and the reaction was stirred for 2 h. The solvent was removed in vacuo, and the residue was purified by silica gel chromatography with 9:1 dichloromethane: methanol as eluent to give *N*-biotinylcysteamine (25 mg, 82 μmol, yield 56%). ESI-MS *m*/*z* $[M+H]^+$ 304.1 $[2M+H]^+$ 607.0.

Preparation of DNA Presenting Monolayers. Self-assembled monolayers (SAMs) of alkanethiolates on gold were prepared as described previously.[27] Briefly, titanium (4 nm) and then gold (80 nm) were evaporated onto glass microscope coverslips using an electron beam evaporator. Monolayers presenting the maleimide group at a density of 5% among tri(ethylene glycol)-terminated alkanethiolates were formed by immersing the gold-coated coverslips in an ethanolic solution of an asymmetric maleimide-terminated disulfide and a symmetric tri(ethylene glycol)-terminated disulfide in a ratio of 1:9 at a total disulfide concentration of 0.5 mM. The monolayers were allowed to form at 4 °C for 24 h, rinsed with ethanol, and dried under a stream of nitrogen. A solution of *N*-biotinylcysteamine (50 μM in PBS, pH 7.2) was applied to the maleimide-terminated monolayers for 4 h at ambient temperature in a humidified chamber. The monolayers were washed with PBS and then water and finally dried under a stream of nitrogen. A solution of streptavidin (0.5 μM, in PBS, pH 7.2) was applied to the biotin-presenting monolayer for 20 min at ambient temperature in a humidified chamber. The monolayers were washed with PBS and water. A solution of 5′-biotinylated oligonucleotide (1 μM in PBS, pH 7.2) was applied to the streptavidin presenting monolayer for 20 min at ambient temperature in a humidified chamber. The monolayers were washed with PBS, followed by water, and then dried under a stream of nitrogen.

For hybridization, a solution of complementary oligonucleotide strand (1 μM in PBS) was applied to the monolayer presenting the immobilized oligonucleotide for 30 min at ambient temperature. The monolayer was then washed with PBS and ammonium citrate (50 mg/ml) and dried under a stream of nitrogen.

SAMDI Mass Spectrometry Analysis. The matrix was applied as a solution (0.3 μL, 5:1 v:v) of 5-methoxysalicylic acid (20 mg/mL in acetonitrile) and ammonium citrate dibasic (50 mg/mL in water) onto monolayers presenting oligonucleotides (∼15 mm^2) and were allowed to dry at ambient temperature. SAMDI spectra were recorded using a Voyager-DE PRO Biospectrometry mass spectrometer (Applied Biosystems, Framingham, MA) with a 337 nm nitrogen laser as the desorption−ionization source. Mass spectra were acquired using a 25 kV accelerating voltage, linear operating mode, and negative or positive polarity. The grid voltage was 95% of the accelerating voltage, and the delay time was 425 ns.

Surface Plasmon Resonance Spectroscopy. SPR experiments were performed on a BIACORE 2000 instrument. A biotin-presenting monolayer was incorporated into the BIACORE cassettes by removing the manufacturer's substrate and applying monolayers using a two-part epoxy (Devcon). PBS was used as running buffer at 3 μL/min. In a typical experiment, a solution of streptavidin (0.5 μM in PBS) was flowed over the monolayer for 20 min followed by buffer for another 20 min. A solution of the biotinylated oligonucleotide (1 μM in PBS) was then immobilized on the streptavidin layer for 20 min and washed. Finally, a solution of the complementary oligonucleotide (1 μM in PBS) was flowed over the immobilized single strand for 20 min and washed. The amounts of DNA bound to the surface or hybridized to the first strand were calculated using the relation 1000 RU = 1 ng/mm^2.

Enzymatic Cleavage of Immobilized Oligonucleotide Substrate. Monolayers presenting 5′-biotin-TTT TTT TTT TTC ACT AT*r*A-GGA AGA G (where rA is riboadenosine) were washed with HBS buffer (10 mM HEPES buffer containing 150 mM NaCl pH = 7.4) and immersed in a solution of the deoxyribozyme (5′-TCT CTT CTC CGA GCC GGT CGA AAT AGT GAA AA, 1 μM in HBS

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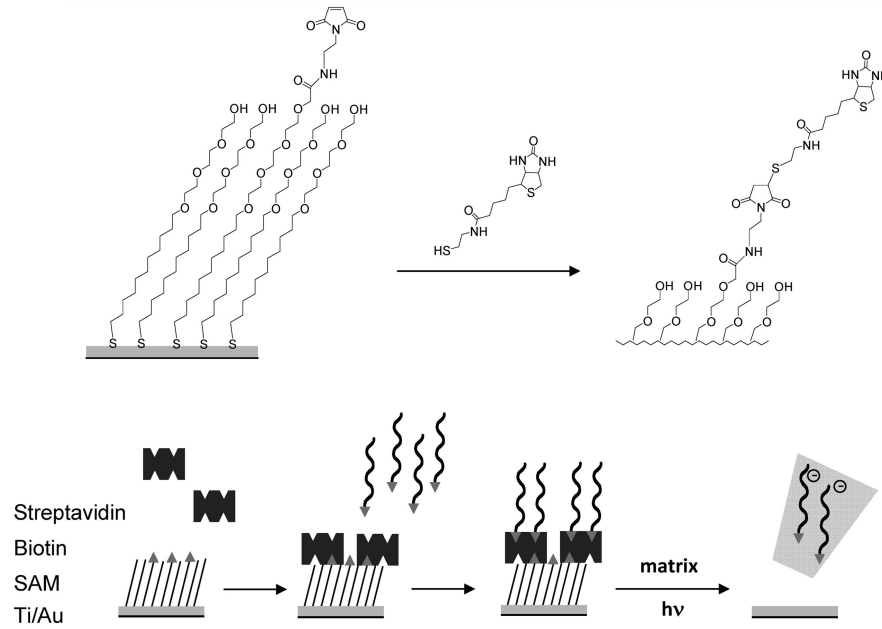

Figure 1. Substrates used in this work were prepared by immobilizing biotin to a monolayer that presents maleimide groups against a background of tri(ethylene glycol) groups (top). Streptavidin was immobilized to the monolayer, allowing the further immobilization of biotinylated oligonucleotides (bottom). Treatment of the monolayer with matrix and analysis with laser desorption−ionization mass spectrometry showed peaks corresponding to the dissociated oligonucleotides.

containing 1 mM $ZnCl_2$) for times ranging from 1 to 90 min at ambient temperature. Reactions were stopped by rinsing the monolayers with solutions of EDTA (5 mM in HBS), HBS, water, and finally ammonium citrate (50 mg/mL). The matrix was applied to each monolayer, allowed to dry, and mass spectra were acquired. The extent of substrate cleavage was calculated from the equation, $100 * [A_{pro}/(A_{sub} + A_{pro})]$ where the peak areas were used to determine the density of substrate (A_{sub}) and product (A_{pro}) ions. All cleavage reactions were performed in triplicate.

Enzymatic Dephosphorylation of Immobilized Oligonucleotide Substrate. Monolayers presenting 5′-biotin-TTT TTT TTT TTC ACT AT*r*AGGA AGA G were treated with the deoxyribozyme (1 μM, 90 min) at ambient temperature as described above. Monolayers presenting the 2′,3′-cyclic phosphate terminated oligonucleotide (5′-biotin-TTT TTT TTT TTC ACT AT*r*A > p) were treated with the T4 polynucleotide kinase (PNK; 10 units/100 μL in 50 mM Tris-HCl buffer, pH = 7.5, containing 10 mM $MgSO_4$ and 5 mM DTT) for 15 min at ambient temperature.[28] The reaction was stopped by rinsing the monolayer with EDTA (5 mM in 50 mM Tris-HCl), 50 mM Tris-HCl, water, and finally ammonium citrate (50 mg/mL). The matrix was applied to each monolayer, allowed to dry, and mass spectra were acquired.

***cis*-Platin Reaction with Oligonucleotide Substrate.** *cis*-Diammineplatinum (II) dichloride (final concentration 112 μM) was added to a solution of 5′-biotin-TTT TAT ATA CGT ATA TCG (200 μL of 5 μM) in sodium perchlorite (10 mM, pH 5.8), and the reaction was incubated at 37 °C for 21 h.[29] Aliquots (5 μL) were taken at defined time intervals and diluted in PBS (10×, 20 μL, pH 7.4), frozen in liquid nitrogen, and kept at −20 °C until analyzed. Frozen samples were thawed and applied on monolayers presenting streptavidin for 30 min, rinsed with PBS, water, and ammonium citrate (50 mg/mL). The extent of oligonucleotide alkylation reaction was calculated by measuring the relative peak area of unmodified oligonucleotide and product ions. All reactions were performed in triplicate.

Results and Discussion

Immobilization of Oligonucleotides. We first immobilized a thiol-tagged oligonucleotide (5′-HS-TTTTTTTTTTCAC-TATAGGAAGAG) to a monolayer that presents maleimide groups against a background of tri(ethylene glycol) groups in a ratio of 1:19. The maleimide group provides for a defined density and orientation of the immobilized oligonucleotide while the tri(ethylene glycol) groups ensure that the monolayers are inert to nonspecific adsorption of molecules in a contacting solution.[30,31] We were unable to observe peaks that correspond to the DNA−alkanethiolate conjugate using any of several matrices, including 3-hydroxypicolinic acid (3HPA), 6-aza-2-thiothymine (ATT), 3,4-diaminobenzophenone (DABP), and 5-methoxysalicylic acid (5MSA) (with ammonium citrate or spermine). We did, however, observe an absence of the peak that corresponds to the maleimide-terminated alkanethiolate and take this as evidence for immobilization of the oligonucleotide to the monolayer. In addition, we could characterize the hybridization of a complementary oligonucleotide to the immobilized oligonucleotide using both surface plasmon resonance (SPR) spectroscopy and mass spectrometry. A control experiment using a noncomplementary oligonucleotide gave no hybridization and rules out nonspecific interactions of the second oligonucleotide with the monolayer. This result is consistent with previous work that has failed to observe covalently immobilized DNA by MALDI-TOF mass spectrometry.[15,16]

We next employed streptavidin-modified surfaces to immobilize oligonucleotides having a 5′ biotin group because this method provides for a stable but noncovalent attachment of the DNA to the monolayer (Figure 1). We started with a monolayer presenting a maleimide group at a density of 5% against a background of tri(ethylene glycol) groups.[27] The monolayer was treated with *N*-biotinylcysteamine, a biotin derivative having a primary thiol, to immobilize the ligand followed by treatment with streptavidin. We used surface plasmon resonance spectroscopy and mass spectrometry to monitor each step in the preparation of the monolayers and subsequent assays of hybridization, enzyme activity, and chemical reactivity (Figure

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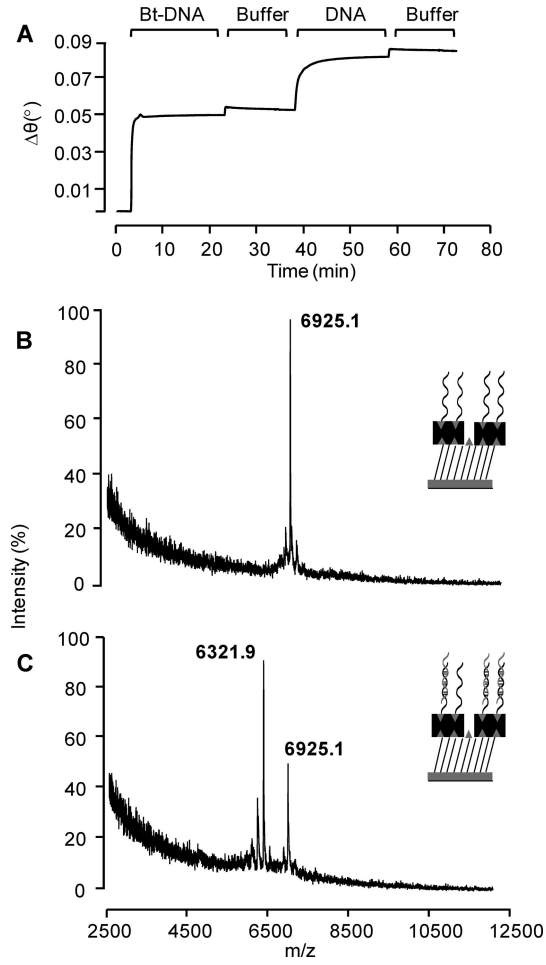

Figure 2. Characterization of the immobilization and hybridization of oligonucleotides on a monolayer. (A) Surface plasmon resonance spectroscopy was used to monitor the immobilization of a biotinylated oligonucleotide (Bt-DNA: 5′-biotin-TTC TTC TCC TTG CTC CTT CTC T-3′) followed by a wash with buffer and then treatment with a complementary sequence (5′-AGA GAA GGA GCA AGG AGA AG-3′). SAMDI TOF MS spectra of the monolayer following immobilization of the first oligonucleotide (B) and hybridization of the complementary sequence (C) reveal clear peaks for each of the strands.

2A). We first used SPR spectroscopy to monitor the immobilization of a biotinylated oligonucleotide (1 μM in PBS) and found that the density of oligonucleotide was 7.9×10^{-12} mol/cm^2 (see the Experimental Section). A subsequent treatment with the complementary sequence led to binding of 5×10^{-12} mol/cm^2 of the complementary strand, representing approximately 60% of the available primary strands.

For mass spectrometry experiments, we rinsed the monolayers with a solution of ammonium citrate (50 mg/mL) to exchange the sodium and potassium ions with ammonium ions and then applied the matrix 5MSA and ammonium citrate and allowed the drops to dry in air. Analysis of the monolayer presenting the single-stranded DNA (5′-biotin-TTC TTC TCC TTG CTC CTT CTC T-3′) revealed a peak at *m/z* 6925.1, corresponding to the biotinylated oligonucleotide that had dissociated from the streptavidin (Figure 2B). An identical monolayer was then treated with a solution containing the complementary 20-mer strand (5′-AGA GAA GGA GCA AGG AGA AG-3′, 1 μM in PBS), rinsed, treated with matrix, and analyzed by mass spectrometry. The spectrum showed two clear peaks at *m/z* 6925.1 and 6321.9 that correspond to the biotinylated oligonucleotide and its complementary strand (Figure 2C). We failed to observe peaks that correspond to noncovalent complexes, either between the two strands of DNA or the biotinylated strand and the streptavidin protein. Control experiments also verified that the immobilization was specific; addition of biotin during immobilization of the capture oligonucleotide or the use of an oligo not having the biotin group both failed to give peaks corresponding to DNA. Finally, we note that we observed strong *m/z* peaks for the oligonucleotides only with the 5-methoxysalicylic acid matrix mixed with ammonium citrate at a ratio of 5:1 and observed low intensity peaks when samples were prepared with the alternate matrices 3-hydroxypicolinic acid, 6-aza-2-thiothymine, or 3,4-diaminobenzophenone.

We do not understand the mechanistic factors that underlie the observation of ions corresponding to the streptavidin-conjugated oligonucleotides, but not those that are covalently attached to the monolayer. In a substantial body of prior work, we have characterized monolayers that were covalently functionalized with a broad range of groups, including peptides, small molecules, carbohydrates, and even proteins, and in all cases could readily observe peaks corresponding to these molecules.[23,31] In any event, we believe that substrates wherein the oligonucleotides are immobilized by way of photolabile tethers or other cleavable functional groups prior to or during the mass spectrometry experiment may also enable the analysis of immobilized DNA strands by MALDI-TOF MS.

Cleavage of Immobilized Oligonucleotide by Deoxyribozyme. We next demonstrate an assay for the cleavage of an immobilized oligonucleotide by a deoxyribozyme.[32–34] A 25-mer biotinylated oligonucleotide strand having a ribose modification at the 18th position was immobilized to a monolayer as described above. The monolayer was treated with a solution of the deoxyribozyme (1 μM, in HBS containing 1 mM Zn^{2+}) for 90 min, rinsed, treated with the 5MSA matrix and ammonium citrate, and analyzed by mass spectrometry. The deoxyribozyme selectively cleaves the immobilized substrate at the single ribose residue (rA) to leave a truncated form of the substrate having a 2′,3′-cyclic phosphate functionality.

A mass spectrum of the monolayer prior to reaction revealed a peak at *m/z* 8087.6 that corresponds to the intact immobilized substrate (Figure 3A). A minor peak at *m/z* 5910.4 was also observed and corresponds to a fraction of degraded substrate that arises either from hydrolysis of the ribose residue or its susceptibility to the laser irradiation.[35] Treatment of the monolayer with the deoxyribozyme (1 μM) for 40 min resulted in an approximately 95% yield of the product as evidenced by the peak at *m/z* 5892.0 (Figure 3B). The extent of reaction was estimated by measuring the peak areas of substrate and product ion and taking the ratio of the former relative to the sum of the peak areas (Figure 3C). We note that the mass spectrometric data does not provide a rigorous quantitative assessment of reaction yields because of differing ionization efficiencies of the monolayer-bound molecules and because of suppression effects. For these reasons, we regard the data provided by SAMDI as semiquantitative.

Removal of 3′-Phosphoryl Group. The enzyme T4 polynucleotide kinase (PNK) acts on substrates having a terminal 2′,3′-cyclic phosphate group to remove the phosphate group.[28] We treated the product of the deoxyribozyme reaction (Figure 3B) with PNK (10 units/100 μL in 50 mM Tris-HCl buffer containing 10 mM $MgSO_4$ and 5 mM DTT) for 15 min and

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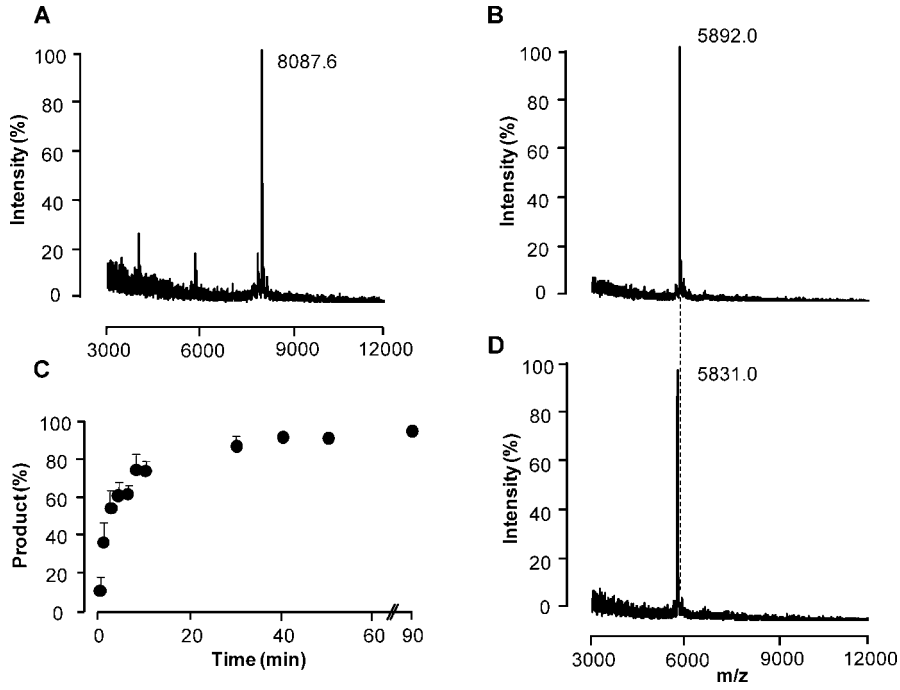

Figure 3. SAMDI-TOF MS quantification of deoxyribozyme (5′-TCT CTT CTC CGA GCC GGT CGA AAT AGT GAA AA) cleavage reaction of immobilized oligonucleotide substrate. Surface-bound biotinylated oligonucleotide (5′-biotin-TTT TTT TTT TTC ACT AT*rA*GGA AGA G) before (A) and after (B) incubation with the deoxyribozyme reaction solution for 90 min. The relative amount of surface-bound product (5′-biotin-TTT TTT TTT TTC ACT AT*rA* > *p*) of the cleavage reaction as function of time as quantified by SAMDI using 5MSA:AC matrix mixture (C). Surface-bound product (5′-biotin-TTT TTT TTT TTC ACT AT*rA)* of the dephosphorylation reaction of 5′-biotin-TTT TTT TTT TTC ACT AT*rA* > *p* by PNK as detected by SAMDI using 5MSA:AC matrix mixture (D).

examined the product by SAMDI MS. The spectra showed a single *m*/*z* peak at 5831.0, which corresponds to the expected product that follows from opening of the cyclic phosphate and removal of this functional group. This example demonstrates the mass resolution inherent to the SAMDI method and the straightforward ability to monitor reactions that cause modest changes to the molecular weight of the nucleic acid substrate.

Reaction of DNA with *cis*-Platin. We next demonstrate an assay of the reaction of *cis*-platin with a single-strand oligonucleotide. *cis*-Platin is an anticancer drug that covalently cross-links DNA inside the cell, causing cell death.[3] Deoxyguanosine is a preferred site for coordination to *cis*-platin on DNA.[37,38] In this example, we demonstrate assays that are performed in solution, that is, using a homogeneous format, and then transferred to a monolayer to selectively immobilize the target oligonucleotides for characterization by SAMDI. This example serves to demonstrate the compatibility of the SAMDI assay with solution-phase assays.

A 5′ biotinylated 18-mer single-strand oligonucleotide (5 μM in 10 mM $NaClO_4$ solution pH = 5.8) having only two deoxyguanine residues, at the 11th and 18th positions, was treated with *cis*-platin (10 eq.) for 21 h at 37 °C. Reaction aliquots (5 μL) were diluted in PBS and applied to monolayers modified with a streptavidin layer for 30 min to immobilize the biotinylated oligonucleotides. The monolayers were then rinsed, treated with matrix, and analyzed by mass spectrometry. *cis*-Platin adducts are expected to result from reaction at the N7 position of the two guanine residues. The SAMDI analysis revealed three *m*/*z* peaks: 5883.2, 6110.5, and 6337.1 corresponding to the original oligonucleotide and the mono- and diplatinum adducts, respectively (Figure 4A−C). Monoadducts were formed during the first three hours, after which the diadducts were apparent (Figure 4D).

The most important result in this work is the development of a method that permits the characterization of immobilized oligonucleotides and of their reaction products by mass spectrometry. The ability to analyze an oligonucleotide tethered to the surface of a biochip—and by straightforward extension, arrays of many oligonucleotides—for any activity that results in a change in mass of the DNA or the presence of molecules that associate with DNA enables the adaptation of oligonucleotide arrays to a broad range of assays that are currently not feasible. Current applications of immobilized oligonucleotides and arrays employ fluorescence detection methods to monitor activities and are almost exclusively applied to characterization of differential gene expression. These assays are straightforward because the target DNA analytes are prepared from polymerase chain reaction using fluorescently labeled primers. Activities other than hybridization can be assayed using oligonucleotide arrays, but the protocol requires a step that introduces a fluorescent label. For example, an antibody having affinity for the intended analyte can be harnessed to screen activities with arrays, as demonstrated by Lutz and co-workers in the identification of 5′-methyl-cytosine residues.[39] In other cases, enzyme or chemical reactions can be used to selectively modify the intended analyte. Deng and co-workers harnessed the dependence of T4 DNA ligase efficiency on mutations to identify gene mutations of rifampin-resistant *Mycobacterium tuberculosis*.[40] The use of mass spectrometry avoids the need for subsequent steps in the assay to introduce the label, and more significantly, enables those assays that are not easily translated into label-based formats. One drawback of

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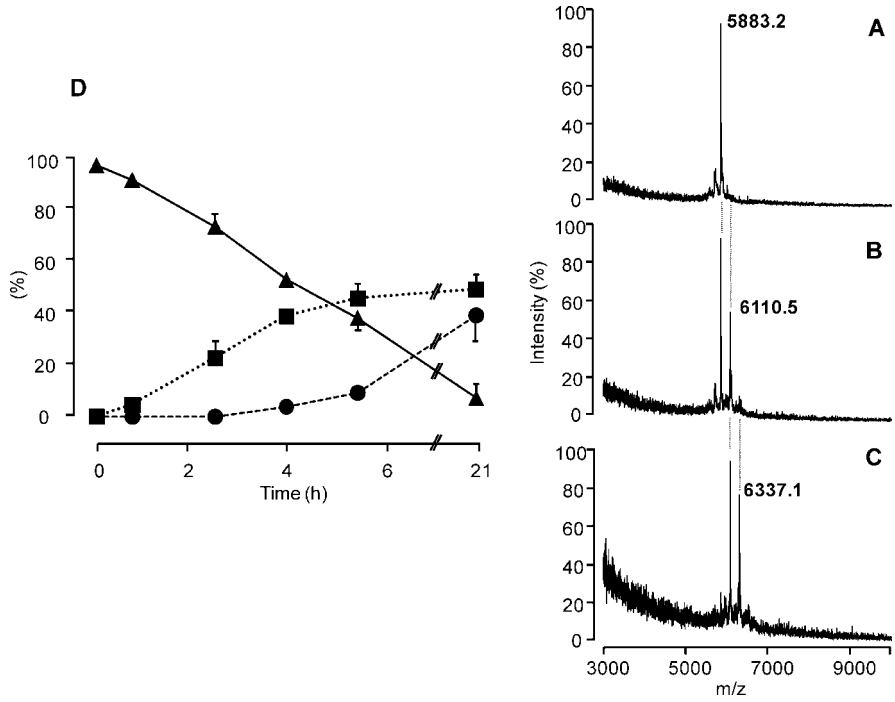

Figure 4. Quantification and analysis of the solution reaction of *cis*-$[Pt(NH_2)_2Cl_2]$ with biotinylated oligonucleotide substrate (5′-biotin-TTT TAT ATA CGT ATA TCG) using SAMDI-TOF MS. Spectra of the captured oligonucleotide from the reaction mixture solution at 0, 4, and 21 h time points (A, B, and C, respectively). Relative amounts of unmodified oligonucleotide sequence (triangular), monoalkylated (square), and dialkylated (circle) reaction products (D).

the mass spectrometry methods, however, is that they have a substantially lower throughput than fluorescence-based assays. Hence, the mass spectrometry assays will not be important for studies of hybridization or other assays that can accommodate the introduction of a fluorescent label.

An important benefit of the SAMDI approach that extends to all immobilized format assays is that it provides a check on the integrity of the immobilized reagents. At times assays fail to provide a signal not because the analyte is absent in the sample but rather because the immobilized reagent is not present or is in an altered form. Grainger, for example, has studied the variability in performance of oligonucleotide arrays and finds that the densities and purities of immobilized reagents are not uniform and give rise to data of varying quality.[41] For oligonucleotides that are synthesized directly on the biochip, it can be difficult to verify that the syntheses proceed with high yield. Because SAMDI provides independent signals for both the capture and the target strands, this technique provides a quality check on the array each time an array is assayed.

This report provides a straightforward strategy for implementing a broad class of assays that use immobilized oligonucleotides. The common use of self-assembled monolayer of alkanethiolates on gold as substrates for the preparation of oligonucleotide arrays[42–44] and the availability of commercial instruments for MALDI-TOF MS that can acquire of order 1000 spectra/h provide a direct route to pursuing applications of arrays of oligonucleotides. The examples in this paper extend the SAMDI MS method to biochemical assays of oligonucleotides, increasing the scope of this method to a broad range of applications in interfacial chemistry and biology.[45]

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