

Detection of Oligonucleotides Hybridized to a Planar Surface Using Matrix-Assisted Laser-Desorption Mass Spectroscopy

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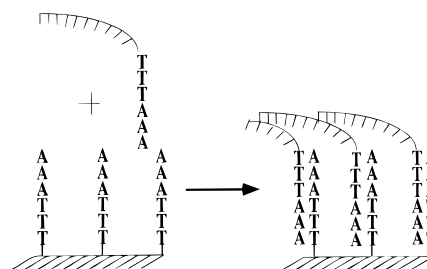
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Abstract: The detection of oligonucleotides hybridized to a planar, plastic substrate using matrix-assisted laser-desorption time-of-flight mass spectroscopy is reported. Oligonucleotides of length 18 to 29 bases were hybridized to a plastic surface upon which a monolayer of an oligonucleotide of sequence 5'-TCC TCT CTC CGT CAT GCG TAT CGT TCA AT-3' was tethered at the 3' end. The target oligonucleotides were hybridized from dilute aqueous solution. The system was prepared for laser desorption by using the matrix 3-hydroxypicolinic acid, deposited at 45 °C. The subsequent mass spectra revealed parent molecular ion for the target DNA with low-level dimerization and no discernible fragmentation.

Introduction

Combinatorial synthetic strategies provide the maximum diversity of chemically distinct products in a minimum number of reaction steps. Parallel synthesis on a planar surface has the additional advantage of providing the combinatorial products in well-defined spatial locations.^{1–3} Arrays of such products have considerable potential for the elucidation of biological recognition mechanisms such as epitope–antibody, oligonucleotide–protein, and oligonucleotide–oligonucleotide interactions. For nucleic acids, the ability to form specific hybridization products, see Scheme 1, on planar combinatorial arrays has led to new diagnostic experiments such as sequencing hybridization.^{4–9} Currently the formation of a complex between a molecule and a biomolecular array is assayed by using either radiography or fluorescence analysis. The ability to chemically differentiate spatially localized hybridization products would lead to a second dimension of analytical information. For instance, a two-dimensional probe would allow implementation of multiplex¹⁰ detection schemes. Here we report an investigation of one example of a chemically distinct detection system, laser-based mass spectral analysis of an oligonucleotide hybridized to a planar substrate. Recent reports reveal that a related experiment, mass spectral analysis of peptides^{11,12} and duplex¹³ DNA bound to microspheres, is possible.

Scheme 1



In the experiment reported in here, an oligonucleotide of known sequence is tethered to a planar plastic surface. A second oligonucleotide (the target oligonucleotide) complementary to the first is allowed to form a complex (hybridize) with the tethered oligonucleotide. The hybridization occurs from a dilute aqueous solution (20 nM) of the target oligonucleotide via Watson–Crick base pairing. This reaction forms a high concentration of the target species at the surface. Since the Watson–Crick base pairing rules are rather stringent, the surface hybridization experiment is quite selective.^{14,15} The formation, or lack of formation, of a specific hybridization product is then assayed in our experiment by using matrix-assisted laser-desorption time-of-flight mass spectroscopy.

Experimental Section

To investigate laser desorption of DNA from hybridization surfaces, we employ a polypropylene plastic surface on which an oligonucleotide of sequence 5'-TCC TCT CTC CGT CAT GCG TAT CGT TCA AT-3' has been synthesized at the 3' end. The polypropylene surface was first derivatized with amine groups in a plasma discharge¹⁶ and then the oligonucleotide was synthesized directly on the amine groups with use of phosphoramidite chemistry.² To conduct a typical hybridization experiment, the oligonucleotide covered surface (the substrate) was first washed with 3.5 M tetramethylammonium chloride (TMACl,^{17–19}

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Aldrich) containing 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, and 0.02 mg/mL SDS. Then a 10- μ L aliquot of a solution containing a total of 100 pmol of target oligonucleotide was added to the substrate suspended in 5 mL of buffer. The final concentration of the oligonucleotide in solution was then 20 pmol/mL. The initial hybridization experiments were allowed to proceed for 7–55 h at room temperature. After hybridization, the substrates were washed for 5–10 min in fresh buffer solution at the hybridization temperature of 25 °C. The resulting hybridization system was then used for laser desorption studies.

Results and Discussion

To determine whether laser-based mass spectroscopy could be used to assay for hybridization the following experiments were performed. The hybridization samples were first prepared for laser desorption/ionization by depositing 1 μ L of a saturated solution of 3-hydroxypicolinic acid (3-HPA) in 50% H₂O:acetonitrile onto the substrate (containing the hybridized oligonucleotide.) The matrix solution was then dried by ramping the substrate temperature from 25 to 45 °C. This resulted in spots with a final diameter of 2 mm. To perform the mass spectral analysis, the hybridization sample was inserted into the laser desorption/ionization region of a time-of-flight mass spectrometer which has been described in detail previously.^{20,21} A 355-nm laser pulse of duration 3 ns and fluence 15–30 mJ/cm² was directed onto the matrix spot and resulting ions were analyzed in the mass spectrometer. Representative mass spectra of the hybridization system under different conditions are shown in Figure 1. Figure 1a represents a control mass spectrum wherein 1 pmol of target 29-mer of sequence 5'-ATT GAA CGA TAC GCA TGA CGG AGA GAG GA-3' in 3-HPA matrix was applied directly to the plastic substrate. The parent ion of the 29-mer has an arrival time of 96.9 μ s (defined at the centroid of the peak), using an extraction voltage of 8 keV. This corresponds to a molecular weight of 9065 u as calibrated by the MW of insulin (5733 u) desorbed from the same surface. The calculated molecular weight of the 29-mer is 9049 u, assuming that all ammonium counterions are converted to H⁺ during laser desorption.²² Figure 1b represents the mass spectrum obtained from the substrate after hybridization of the complement from dilute solution as described previously. The arrival time of the hybridized target is identical with that for the control shown in Figure 1a. The mass spectral signal from the hybridized target strand is stable and highly reproducible. An upper limit of 1 pmol of target strand has been measured^{23,24} on such polypropylene supports by using dimethoxytrityl analysis.

To further characterize the identity of the species giving rise to the mass spectral feature at m/e 9065 u, a number of control experiments were performed. It is possible that the tether oligonucleotide was laser desorbed and detected. Depositing matrix on a substrate lacking the target oligonucleotide, followed by otherwise identical processing before laser desorption, produced no mass spectral signal. Note that in Figure 1c, no mass spectral signal is observed for the tethered oligonucleotide. One other possible scenario for producing the mass spectrum

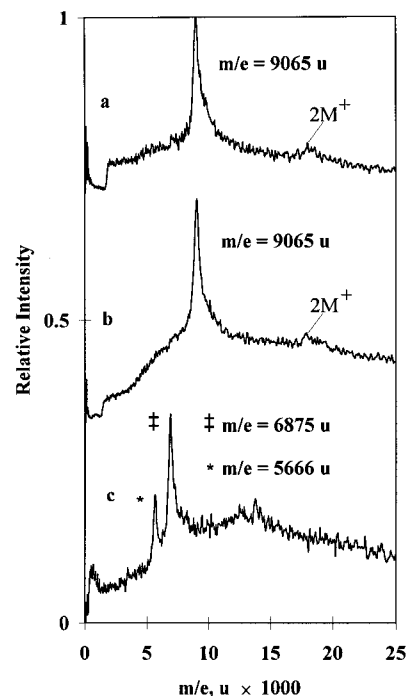


Figure 1. Matrix-assisted laser desorption time-of-flight mass spectra for (a) 1 pmol of target 29-mer in 3-HPA matrix when applied directly to the plastic substrate, (b) target 29-mer hybridized onto the substrate from a dilute solution (20 nM), and (c) a mixture of target 18-mer and 22-mer hybridized from dilute solution with subsequent processing as described in the text. Note that in spectra a and b the ion intensity between 0 and \sim 2000 u is suppressed by using a pulsed electrostatic deflection system.

in Figure 1b would be nonspecific binding of the target strand to the substrate rather than specific hybridization through Watson–Crick base pairing. To test for nonspecific binding we investigated whether a noncomplementary 25-mer and 45-mer would bind and subsequently provide a mass spectral signal. We observed no signal for either of the noncomplementary strands. We note that the direct application of the noncomplementary 25 and 45-mer in matrix (1:10000 molar ratio) to the substrate results in readily observable mass spectral peaks at 7654 and 13945 u, respectively. Subsequent experiments using the complementary 29-mer revealed mass spectral signal after 7 h of hybridization, suggesting that even shorter hybridization times will result in detectable signal. Mass spectral signal was also observed after the hybridization complex had been stored at 4 °C in air for 6 weeks. This suggests that the hybridization system is robust. Finally, a mixture of two shorter complementary strands 5'-CGC ATG ACG GAG AGA GGA-3' and 5'-GAT ACG CAT GAC GGA GAG AGG A-3' was hybridized from dilute solution. The target strands were laser desorbed and mass analyzed to produce the spectrum shown in Figure 1c. The features observed at 5666 and 6875 u correspond to the 18-mer (calculated molecular weight 5623 u) and the 22-mer (calculated molecular weight 6883 u), respectively. The calculated molecular weights were again based on conversion of counterions to H⁺.

The ability to mass spectrally detect an oligonucleotide hybridized to a plastic substrate suggests that chemically selective detection of hybridization arrays is now possible. The advantage of the laser-based mass spectral detection is that two dimensions of information can be obtained from the hybridization array. The first dimension is the location of positive binding events. These "pixels" can be assayed with high resolution \sim 50 μ m. Similar assays can also be performed by using radiographic (300 μ m) and fluorescence detection (50 μ m) currently. The

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second dimension of information in such an experiment stems from the mass spectrum associated with the hybridization pixel. It is of value to note that the laser-based mass spectroscopic method usually results in limited observation of oligonucleotide fragmentation when a linear time-of-flight configuration is employed.²⁵ This should lead to a significant multiplex advantage for array analysis. For instance, a 3×3 array may have up to 2^9 distinct combinations when radiographic analysis is employed. A mass spectral scheme analyzing 10 different masses would result in 10^{27} distinct combinations. The

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combination of these two detection dimensions should result in significant advances in diagnostic capabilities.

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