OLIGONUCLEOTIDE-MODIFIED SURFACES FOR AFFINITY-MALDI-TOF-MS OF PROTEINS AND BIOLOGICAL SAMPLES

by

Jacquelyn Rose Cole

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Examining Committee:

Linda B. McGown, Thesis Adviser
Wilfredo Colón, Member
Mark Platt, Member
Jonathan Dordick, Member

Rensselaer Polytechnic Institute
Troy, New York

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ABSTRACT

Previous work demonstrated the use of aptamer-modified surfaces for affinity-MALDI-MS using the model system of thrombin capture by the thrombin-binding aptamer and the use of genome-inspired oligonucleotides in affinity-MALDI-MS using the insulin-linked polymorphic region to capture insulin.

The first part of this dissertation describes in depth characterization of the affinity-MALDI-MS platform for the capture and detection of immunoglobulin E (IgE) in simple solution and in human serum using an aptamer-modifed probe surface. Detectable signals were obtained for 1 amol of IgE applied either in a single, 1 μL application of 1 pM IgE or after 10 successive, 1 μL applications of 100 fM IgE. In both cases, the surface was rinsed after each application of IgE to remove sample concomitants including salts and free or nonspecifically associated proteins. Detection of native IgE, which is the least abundant of the serum immunoglobulins and occurs at subnanomolar levels, in human serum was demonstrated and interference from the high abundance immunoglobulins and albumin was investigated. The aptamer-modified surface showed high selectivity toward immunoglobulins in serum, with no significant interference from serum albumin. Addition of IgE to the serum suppressed the signals from the other immunoglobulins, confirming the expected selectivity of the aptamer surface toward IgE. Dilution of the serum increased the selectivity toward IgE; the protein was detected without interference in a 10 000-fold dilution of the serum, which is consistent with detection of IgE at amol (pM) levels in standard solutions.

The second part of this dissertation describes the use of the affinity-MALDI-MS platform for the discovery of protein biomarkers using genome-inspired oligonucleotides

as the affinity-binding agents. The oligonucleotide sequences were derived from promoter regions of several genes, including the insulin-linked polymorphic region, the c-MYC oncogene promoter region, the vascular endothelial growth factor promoter region, and the retinoblastoma promoter region. These oligonucleotides, all of which form G-quadruplex structures, were used to capture proteins from human serum and nuclear extracts. G-quadruplex forming oligonucleotides with sequences derived from genomic DNA have been found to exhibit high affinity towards target molecules including proteins and can be employed as affinity agents in a manner analogous to aptamers, which are combinatorially selected oligonucleotides,

Protein capture using oligonucleotide-coated beads was also investigated in order to facilitate capture of proteins for further analysis. Coupling the information gained through the affinity-MALDI-MS technique with the off-line collection of the molecules for further identification and quantification could revolutionize the way proteomic analyses are performed.

The dissertation also describes the characterization of the oligonucleotide-modified fused silica surfaces through the use of atomic force microscopy. Two surfaces were used, the IgE aptamer-modified surface and the c-MYC-modified surface. Images were obtained of the bare fused silica surface, and of the oligonucleotide-modified surfaces before and after protein capture and after cleaning.