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Immobilization-mediated reduction in melting temperatures of DNA–DNA and DNA–RNA hybrids: Immobilized DNA probe hybridization studied by SPR

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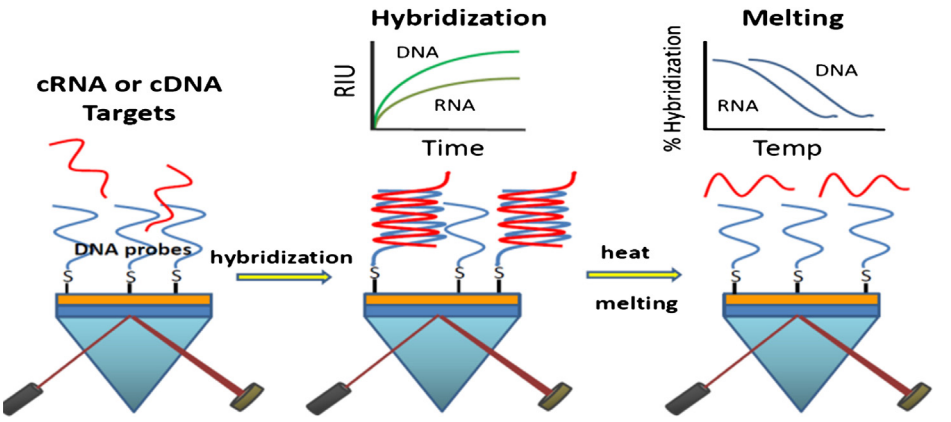
HIGHLIGHTS

- We report hybridization of DNA and RNA to a surface-bound DNA probe derived from TMV using SPR.
- For both RNA and DNA, the melting temperatures were ~20 °C lower for solid state than in solution.
- Short oligomeric RNA targets may require measurement temperatures below room temperature.
- The melting temperature of the DNA:RNA hybrid was 17 °C lower than DNA:DNA duplex in the solid state.
- Hybrid melting temperatures are critical variables for biosensor oligonucleotide analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

The melting temperature of probe-target nucleic acid hybrids plays an important role in sensor performance. This study employed surface plasmon resonance (SPR) techniques to investigate the hybridization of both DNA and RNA targets to their complementary surface-bound DNA oligonucleotide and the corresponding melting temperatures. The target molecule was a 26 nucleotide strand of RNA selected because of its utility in detecting the tobacco mosaic virus, a common plant pathogen with an RNA genome. The melting temperatures of duplexes with immobilized probes were determined by hybridization of the DNA and RNA targets with the bound DNA probe at different temperatures using a custom-built thermostated dual-channel SPR cell. Hybridization conditions and binding efficiencies were compared for both DNA and RNA binding to the gold-coated surface-bound DNA probe. The melting temperature for the DNA–DNA duplex was approximately 15 °C higher than the DNA–RNA in the solid state. The melting temperature in solution was also measured for comparison to the surface values. For both RNA and DNA, the melting transitions were substantially lower (~20 °C) for solid state binding relative to solution. Particularly for surface immobilized DNA–RNA hybrids, the melting temperature was reduced to

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room temperature or below. Since most biosensor platforms operate at room temperature, they may consequently exhibit poor sensor performance due to weakened probe-target interactions. This result emphasizes that for optimal sensor performance, the hybrid melting temperature should be considered. The ability to study and optimize the binding of complementary strands of genomic materials to bound DNA probes could facilitate the rapid detection and identification of plant viruses having genomic RNA using various biosensor rapid analytical techniques.

1. Introduction

The ability to rapidly detect and identify biomolecular markers specific to certain microorganisms would provide significant advances to human health, environmental stewardship, and agriculture. For example, current standard methods for identification and quantification of bacterial pathogens rely on culturing and colony counting. This method is sensitive, but can take days to weeks to perform. Immunological-based techniques reduce the analysis time substantially, but are still incapable of real time analysis, are labor intensive, often suffer from limited sensitivity and selectivity, and are sometimes complicated by contaminants. Polymerase chain reaction (PCR)-based amplification of isolated genomic fragments is faster than culturing (5–24 h vs days), but suffers from time-consuming sample preparation and purification that rely on intensive manual labor and expensive reagents. These limitations have led to an explosion of biosensor techniques targeting rapid pathogen identification [1–6]. Among these sensing modalities, electrochemical [7,8] and molecular conductance [9] measurements based on hybridization of pathogen or other organismal DNAs to specific complementary DNA probes are interesting because of their high sensitivity. However, for pathogens whose genomes are composed of RNA (i.e. having no DNA component in replication), such as most plant viruses, these new methods should target RNA instead of DNA. In addition, RNA exhibits several important advantages, including (i) biological amplification within the organism thereby removing the need for further nucleic acid amplification and (ii) providing insight into the temporal state of the cells (e.g., gene expression) in contrast to the solely static information provided by DNA. It is therefore highly desirable to directly sense RNA molecules in order to simplify microbial detection and identification. While the effects of varying salt concentrations on the melting temperature for DNA:DNA duplexes are well-known [10–12], less understood is the role of hybridization temperature and sensing modalities based on DNA:RNA hybrids, particularly on solid state surfaces that display DNA probes. Such differences between DNA:DNA duplexes vs DNA:RNA hybrids have been observed in solution, where DNA:RNA hybrids exhibited lower melting temperatures (T_m) in aqueous solutions [13,14].

To determine how hybridization conditions vary between a DNA:RNA hybrid and a DNA:DNA duplex of the same sequence, we studied the melting temperature for a specific 26 nucleotide sequence derived from the plant pathogen *Tobacco mosaic virus* (TMV). TMV was selected for this initial study due to the high levels of TMV RNAs found in infected plant tissues, the ultimate targets for RNA detection. TMV produces both genomic RNA for packaging in virons and for production of its replicase proteins and subgenomic RNAs for production of its movement and coat proteins which are needed at higher concentrations later in the infection cycle [15]. The subgenomic RNAs responsible for the production of movement and coat proteins represent the 3′ terminal 25% and 11% of the genomic RNA, respectively. As the 26-mer RNA selected here is found near the 5′ end of the genetic RNA, it is present only in the lower concentration, full length TMV RNA.

We chose surface plasmon resonance (SPR) to identify the optimal temperature conditions for both DNA and RNA hybridization to a complementary surface-bound DNA probe. SPR has been well established as a label-free method for real-time monitoring the change in refractive index at the interface as a target in solution binds to a ligand tethered to a solid support. In this study, the hybridization of a target TMV 26 nucleotide RNA (RNA 26-mer) and a target DNA with the same 26 nucleotide sequence (DNA 26-mer) to a complementary surface-bound DNA probe was monitored by SPR and the respective melting temperatures were studied. To the best of our knowledge, no SPR determination of a DNA:RNA hybrid melting temperature and comparison with the respective DNA:DNA duplex on solid supports has been reported.

In addition to the variable temperature studies in the solid state, similar studies were also performed in solution for comparison. We demonstrate that the melting point is significantly depressed from the solution phase T_m when the probe molecules are bound to the surface. These experiments demonstrate that hybridization conditions and the subsequent effect must be an important consideration in the design of oligonucleotide detection systems. SPR studies provide valuable input for study of hybridization conditions and subsequent duplex melting in the development of new solid state sensing methods for sensitive and direct target RNA detection.

2. Material and methods

2.1. Materials

In this paper, we refer to the complementary strands for hybridization to bound substrate probe DNA as target DNA and RNA. Selection of the 26-mer RNA sequence used here was based on in silico analysis of the TMV genome sequence simulating ribonuclease T1 (RNase T1) digestion (cleavage after G residues) followed by sorting of the fragments by size. RNase T1 digestion was selected because it can yield a population of RNAs with more controlled sizes defined by the distribution of G residues within the degraded RNA. The 26-mer TMV sequence used was chosen both for its length and GC content. It is also sufficiently long that it is unlikely that its sequence would be found in the transcriptosome of uninfected plants and presumably is long enough for stable hybridization at room temperature or above.

The DNA probe, a thiolated 26 nucleotide precursor (SH-26-mer) (provided as the disulfide), and the target sequence RNA and DNA 26-mers used in this study were synthesized by Integrated DNA Technologies (Coralville, Iowa). Their sequences are as follows.

- DNA probe SH-26-mer disulfide precursor: 5′ -S-S-C_6-CGTGTTATAAAATGTAATTTGGAATT 3′
- Target RNA 26-mer: 5′ AAUUCCAAAUUACAUUUUAUAACACG 3′
- Target DNA 26-mer: 5′ AATTCCAAATTACATTTTATAACACG 3′

Ethylenediamine tetraacetic acid (EDTA), sodium phosphate dibasic (Na_2HPO_4), 5 M NaCl, 1 M KCl $MgCl_2$, and glycine were purchased from Sigma–Aldrich (Milwaukee, WI). MeO-PEG-SH (MW 740) was obtained from Iris Biotech (Santa Clara, CA). Bond-Breaker TCEP (tris(2-carboxyethyl)phosphine) Solution, Neutral pH, used for reduction of the disulfide, was purchased from ThermoFisher