A Fiber-Optic Evanescent Wave DNA Biosensor Based on Novel Molecular Beacons

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We have prepared a novel optical fiber evanescent wave DNA biosensor using a newly developed molecular beacon DNA probe. The molecular beacons (MB) are oligonucleotide probes that become fluorescent upon hybridization with target DNA/RNA molecules. Biotinylated MBs have been designed and immobilized on an optical fiber core surface via biotin–avidin or biotin–streptavidin interactions. The DNA sensor based on a MB does not need labeled analyte or intercalation reagents. It can be used to directly detect, in real-time, target DNA/RNA molecules without using competitive assays. The sensor is rapid, stable, highly selective, and reproducible. We have studied the hybridization kinetics of the immobilized MB by changing the ionic strength of the hybridization solution and target DNA concentration. Our result shows divalent cations play a more important role than monovalent cations in stabilizing the MB stem hybrids and in accelerating the hybridization reaction with target DNA/RNA molecules. The concentration detection limit of the MB evanescent wave biosensor is 1.1 nM. The MB DNA biosensor has been applied to the analysis of specific γ-actin mRNA sequences amplified by polymerase chain reaction.

Hybridization of nucleic acids to their complementary sequences is a fundamental process in cellular and molecular biology and has been the key for the understanding of many biological processes and for biotechnology development. There have been many different methods developed for its study.1–15 Traditional methods to study DNA hybridization are usually slow, requiring hours to days to produce reliable results.1 Biosensors offer a promising alternative for faster hybridization assays. There are many interesting applications of DNA biosensors in clinical diagnostics, forensic identification, and a host of biomedical research areas.2,3 The DNA biosensors identify specific target DNA or RNA molecules on the basis of the complementary coupling between specific nucleotide sequences immobilized onto a solid support (i.e., transducer). Currently, these sensors are largely based on surface acoustic waves,4 electrochemical detection,5 and optical transducers.6–14

DNA biosensors based on optical and spectroscopic measurements form one of the most promising families of bioaffinity transducers. There are several types of optical DNA biosensors based on surface plasma resonance (SPR)6 and optical fibers.8–14 Optical-fiber biosensors offer several advantages over other biosensors: small size, flexible geometry, remote working capability, and noise immunity10–14 which make them ideal for clinical applications, environmental monitoring, and process control with easy operation. The fiber-optic evanescent wave biosensor is based on total internal reflection, a fundamental characteristic of an optical fiber. A small portion of the light traveling in a fiber core penetrates into the surrounding medium, with the intensity of the evanescent field decaying exponentially from the fiber core surface into the medium. The evanescent wave excites fluorophores immobilized on or flowing around the core surface. Fluorescence produced from the fluorophores is coupled back into the fiber core for transmission to an optical detection system. This approach avoids laborious optical adjustment and has the potential of being adapted to other measuring devices. Optical-fiber-based biosensors are thus convenient and effective in practical applications. Many biomolecule-based evanescent fiber sensors, especially those based on DNA and proteins, have been developed for sensitive detection and biomolecular interaction studies.15 The major advantages of evanescent wave biosensors are low background, low noise, remote working geometry, and the capability of monitoring surface interactions which have been explored in living cell membrane studies.

There have been several optical fiber evanescent wave DNA sensors.11–13 Most of the evanescent optical DNA sensors use one

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of the following three signal transduction mechanisms: labeled target DNA molecules,11,12 intercalation reagents,13 or competitive assay.11 To detect an unlabeled complementary 16-mer oligonucleotide, competitive hybridization assays were performed with a fiber-optical evanescent wave DNA sensor, resulting in a detection limit of 1.1 nM of 16-mer fluorescein-labeled oligonucleotides.11 Pilevar et al.12 presented a hybridization assay that relies on the evanescent wave excitation of optical-fiber surface-bound fluorophores. Short oligonucleotides (20-mer) bound to the fiber surface have been used to detect near-IR dye-labeled complementary sequences at the subnanomolar levels. H. pylori total RNA had been detected by the biosensor in a sandwich assay. Piunno and Krull13 covalently immobilized oligonucleotide probes onto an optical-fiber surface for DNA detection. The hybridization on optical fibers was monitored by the use of the fluorescent DNA stain reagent, ethidium bromide. Even though these biosensors are remarkable in detection capability and useful for some interesting applications, there are several critical limitations due to the need for labeled targets or the use of a fluorescent DNA stain reagent, i.e., an intercalation reagent, or the need for a competitive assay. Therefore, it is difficult to perform real-time hybridization studies and to quantitatively monitor hybridization kinetics on the surface and impossible to study biological processes in real time and in vivo.

In this paper, we describe the development of an optical-fiber evanescent wave DNA biosensor based on a newly synthesized molecular beacon DNA probe.14 Molecular beacons (MB) are a new class of oligonucleotides that can report the presence of specific nucleic acids in homogeneous solutions. The MB probe consists of a stem-and-loop structure. The loop portion of the molecular beacon is a probe sequence complementary to a predetermined target DNA. The stem is formed by the annealing of two complementary arm sequences that are on either side of the probe sequence. A fluorescent moiety is attached to the end of one arm and a nonfluorescent quenching moiety is attached to the end of the other arm. The stem keeps these two moieties in close proximity to each other, so that the fluorescence is quenched by energy transfer. When the loop sequence hybridizes to the target sequence the stem opens and the fluorophore separates from the quencher in the stem, leading to the restoration of the fluorophore’s fluorescence. Recently, a biotinylated ssDNA MB has been designed for DNA hybridization studies at a liquid—solid interface and for the development of ultrasensitive DNA sensors.15 In this study, the biotinylated ssDNA MB will be immobilized on an optical fiber core surface through a biotin—avidin binding immobilization method. The evanescent wave generated at the core surface of the fiber is used to excite the fluorophores on the fiber surface, creating a fiber-optic evanescent wave DNA biosensor. The DNA sensor is used to quantitatively detect its target DNA or RNA samples and to study the hybridization kinetics of the immobilized MB.

**EXPERIMENTAL SECTION**

**Materials.** We have designed two different biotinylated molecular beacons and their target oligonucleotides: molecular beacon A, 5′-[(TMR) CCT AGC TCT AAA TCG CTA TGG TCG]′] and molecular beacon B, (5′-TM R-GCACG TCC ATG CCC AGGG AAG GAA GG (Biotin dT) GC-DABCYL-3′). The biotinylated ssDNA molecular beacon was labeled with tetramethylrhodamine (TMR) as the fluorophore and DABCYL (dimethylaminoazobenzene aminoexal-3-acyanido) as the quencher. Unless otherwise specified, all the experiments carried out in this paper were done with molecular beacon A. The mRNA sample was synthesized locally. Affinity-purified avidin and streptavidin were purchased from Sigma (St. Louis, MO). Sulfosuccinimidyl-4-(biotinamido) hexanoate (NHS-LC-biotin) were obtained from Pierce (Rockford, Illinois). Trimethoxysilylpropyldiethylamidin (DETA) was purchased from United Chemical Technologies (Bristol, PA). Superpurified water was used for the preparation of all solutions. Silica optical fibers with a core diameter of 105 μm were obtained from General Fiber Optics, Inc. (Fairfield, NJ). All reagents were used without further purification.

**Preparation of a Molecular Beacon DNA Sensor.** A batch of 8 optical fibers of 105 μm diameter was used in a single immobilization cycle. About 2 cm of cladding was stripped away from the core by chemical etching at one end of the fiber probe. The fiber probe was perpendicularly dipped into a 49%hydrofluoric acid solution for 12 min. The HF solution was covered by heptane solvent. Under our experimental conditions, the fiber probe with a core diameter of ~60 μm was obtained to best couple the fluorescence signal by avoiding signal loss from V-number mismatch between the MB-coated section and the cladded fiber.16 The etched fiber probe was sufficiently washed with water before being used for the subsequent immobilization experiment.

Biotinylated MBs were immobilized on the etched portion of the fiber for DNA sensing (Figure 1). The etched fiber probes were first cleaned by immersion in a 1:1 v/v concentrated HCl/MeOH mixture for 30 min, rinsed in water, and submerged in concentrated sulfuric acid for 30 min. Further rinsing and then boiling in water for 8–10 min followed. Silanization of the fibers was performed by immersing them in a freshly prepared 1% (v/v) solution of DETA in 1 mM acetic acid for 20 min at room temperature (23 °C). The DETA-modified fiber probes were thoroughly rinsed with water to remove excess DETA. The silanized fiber probes were dried under nitrogen and fixed by

![Figure 1](image.png)
heating in a 120 °C oven for 5 min. Then, the silanized fibers were immersed in 0.5 mg/mL NHS-LC-biotin in 0.1 M bicarbonate buffer (pH 8.5) for 3 h at room temperature. Both avidin and streptavidin were used in our experiments. We will mainly discuss the results of streptavidin in the following sections. Streptavidin was bound to the fiber surface by incubating the biotinylated fibers overnight at 4 °C in a solution containing 1.0 mg/mL of streptavidin. The streptavidin-immobilized optical fibers were immersed with a biotinylated MB solution (1 × 10⁻⁶ M in 10 mM phosphate buffer (pH 7.0)) for as long as 20 min or overnight at 4 °C to allow the biotinylated MB to be immobilized on the surface. The fiber probes were stored in 10 mM phosphate buffer at 4 °C for future use.

RNA Sample Preparation. Total RNA was isolated from rat lung tissues and then reverse transcribed (RT) to first-strand cDNA with oligodT primer by using a cDNA cycle kit according to the manufacturer’s instructions (Invitrogen BV). The RT products was used as a template for PCR. PCR was performed for 25 cycles using oligonucleotide 5’-GCC CCT CCG GTG TCC AGA-3’ and 5’-GCC AGG GCT GTG ATC TCC-3’ as upper primer and lower primer, respectively. Two hundred bp DNA A fragment was produced by the PCR amplification and cloned into the PCR2.1 vector (TA cloning Kit, Invitrogen). The recombinant plasmid was transformed to Escherichia coli cells. M inepres DNA was carried out and linearized with BamHI. A typical T7 transcription reaction containing 1.0 μg of linearized plasmid DNA template was carried out by using the Ambion Megascript in vitro Transcription Kit. A 204-nr nt γ-action RNA was produced and purified.

Optical Instrumentation. The experimental setup used for the DNA evanescent wave sensor characterization was based on an inverted Olympus fluorescence microscope (IX70-S8F). In the apparatus, a photomultiplier tube or an ICCD (Princeton, EEV 512 × 1024 FT) was mounted on the top entrance port of the microscope for optical detection and imaging. The source for the excitation radiation was a 514-nm laser beam from an Innova 307 Ar⁺ laser (Coherent Laser, Santa Clara, CA). The proximal end of the DNA fiber sensor was put in a sample cell containing the hybridization buffer. An evanescent wave field was generated on the surface of the exposed optical fiber core and used to excite the fluorophores immobilized on the longitudinal surface of the fiber. The resulting fluorescence signal was monitored through the same fiber. To specifically monitor the MB fluorescent signal, a 550-nm-long pass filter and a 580-nm interference filter were put in front of the optical detector. The whole setup was put on a floating laser table for vibration isolation.

Hybridization of Target DNA/ RNA to an Immobilized MB on an Optical Fiber Surface. The sensor surface was washed with hybridization buffer (20 mM Tris-HCl, pH 8.0, 0.5 M NaCl). Complementary oligonucleotide or RNA sample was added, and the sensor response was monitored over time. To minimize photobleaching, a shuttered excitation source was applied during data acquisition. We used initial reaction rates to characterize the sensor’s activity, which were calculated using the linear portion of the hybridization dynamic curve over time.

RESULTS AND DISCUSSION

Immobilization of a Molecular Beacon on an Optical Fiber Surface. The two most common methods for DNA immobilization onto a solid surface are covalent attachment and avidin–biotin binding. It has always been an advantage if the DNA can be attached to a solid surface with a spacer connected to one of the ends of the nucleic acid chain. This spacer leads to DNA molecular flexibility. Since 5′ and 3′ ends of the MB are linked to a fluorophore and a quencher, respectively, the avidin–biotin binding method is used for MB immobilization. The biotin was connected to a dT by a spacer to increase the degree of freedom for MB hybridization. We chose to link biotin to the quencher side of the stem to minimize potential effects the immobilization might have on the fluorophore’s fluorescence.

The MB immobilization method is specifically designed for the MB sensor preparation by combining several surface modification techniques. The procedure is schematically depicted in Figure 1. The immobilization process consists of a few important steps: silanization which adds amine groups (–NH₂) to the surface; conjugation between the amine groups and the NHS-ester of biotin (NHS-LC-biotin) with a spacer arm of 22.4 Å, yielding a stable amide bond; and coupling avidin to the biotinylated surface. As shown in Figure 1, the streptavidin serves as a bridge between the surface-immobilized biotin and a biotinylated MB. The spacer (LC) reduces steric hindrances associated with biotinylated MBs. The streptavidin–biotin binding process was monitored in real time by incubating the streptavidin-immobilized fiber probe with 1 μM biotinylated MB. The process was fast and completed within a few minutes. The streptavidin–biotin binding on the fiber surface was also stable and reproducible. There was no significant leakage of the immobilized MB when immersed inside buffer solution even after 10 days. The surface density of the immobilized MB on the probe is an important parameter in the hybridization reaction. In the following experiments, all probes have equilibrium MB coverage. Our results showed that the probes prepared with equilibrium coverage were reproducible.

Detection of One-Base-Mismatch DNA. The hybridization of three oligonucleotides to the MB immobilized on the optical fiber surface was investigated. A real-time dynamic process of the hybridization was followed. Figure 2 shows the hybridization process of the MB evanescent wave biosensor with complementary oligonucleotide (5’-GCC ACC ATA GCG ATT TAG A-3’), one-base-mismatch oligonucleotide (5’-GCC ACC ATA TCG ATT TAG A-3’), and noncomplementary oligonucleotide (5’-GCC ATG GTG TGG ACA TTC A-3’). There was no increase in fluorescence intensity for noncomplementary DNA. Within 1 min upon addition of complementary oligonucleotide, a significant increase in fluorescence intensity was observed. The hybridization to target DNA was rapid and nearly completed within 10 min. When the one-base-mismatch DNA was used, a smaller increase of fluorescence intensity was observed. This indicates that the duplex formed is destabilized by even a single mismatched base. Even though there is only a 1.8 times better selectivity for the perfectly matched DNA at the equilibrium state, the difference at the early stage of hybridization is much larger (4.3 times at 3 min). This is critically
of the mismatched bases in the sequence all have strong effects on the hybridization sequence, the GC contents, and the location of the mismatched bases in the sequence all have strong effects upon duplex stability.\textsuperscript{23} The specificity of the MB is higher than that for a linear probe due to the existence of the MB's hairpin stem structure.\textsuperscript{24} Tyagi and Kramer\textsuperscript{16} reported that there was no fluorescence in the presence of a one-base-mismatched oligonucleotide. Therefore a MB sensor with even higher specificity is obtainable by careful designing of the loop length, the base composition, and the position of the mismatched base within the hybridization sequence.

Chemical Regeneration of the MB Biosensor. Reusability has always been a desired feature for biosensors in practical applications. Usually the regeneration of a DNA sensor was achieved by either a thermal or a chemical method.\textsuperscript{11} Considering that the avidin layer might be destroyed at high temperature, we used a chemical method to regenerate the evanescent wave DNA sensor. The MB sensor can be regenerated by immersing it in 90% formamide in a TE buffer (10 mM Tris-HCl, pH 8.3, 1 mM EDTA) for 1 min at room temperature. After regeneration, the MB sensor returned to its original state, indicating that the double-stranded DNA hybrid was dissociated into single strands and the stem of the immobilized MB was closed again. The regenerated MB sensor produced a similar fluorescence increase when it was hybridized to its target, demonstrating the regeneration of the MB sensor. The regeneration is less successful after a few repeated assay cycles. We believe that the major problem is the restoration to the original single-stranded DNA MB form after repeated uses. Improvement in MB immobilization and regeneration conditions will have to be worked out for long-time application of the DNA sensor.

Effect of Ionic Strength on the Kinetics of MB Hybridization on a Sensor Surface. Kinetics studies of nucleic acid hybridization are necessary for understanding this important biological process on a molecular level. The hybridization between the immobilized MB and its target oligonucleotide on the fiber surface was studied in 4 different ionic environments. We maintained the same ionic strength (I) environment for all cation solutions (I = \( \frac{1}{2} \sum Z_i^2 C_i \)), \( C_i = \) ionic concentration, \( Z_i = \) ionic charge.

Table 1 shows the influence of different cations on the stem stability of the immobilized MB probe itself. The fluorescence intensity of the MB is due to incomplete quenching.\textsuperscript{16}–\textsuperscript{17} The more stable the stem structure in a MB, the lower the fluorescence intensity of the MB probe in the presence of divalent cations is compared to its presence in the presence of monovalent ions. Figure 3 shows the time course of the hybridization reaction of the immobilized MB to its complementary oligonucleotide on the optical fiber surface with different cations and the same ionic strength in the solution. Hydration rates could hardly be observed without salts. In the presence of divalent cations, the hybridization rates increase greatly. The initial reaction rate in the presence of divalent cations is \textasciitilde 20 times higher than that in the presence of monovalent ions at the same ionic strength, suggesting that divalent cations play a more important role in decreasing electrostatic repulsion between anionic chains of MBs on the surface and the target nucleic acids in the solution. The screening of the electrostatic charges associated with bases stabilizes both the stem structure

![Figure 2](image)

**Figure 2.** Dynamics of hybridization of MB evanescent wave sensor: (a) 100 nM noncomplementary oligonucleotide; (b) 100 nM one-base-mismatched oligonucleotide; (c) 100 nM complementary oligonucleotide. All experiments were performed in a hybridization buffer of 20 mM Tris-HCl, pH 8.0, 0.5 M MgCl\(_2\).

![Figure 3](image)

**Figure 3.** Effect of metal ions on the hybridization reaction of the immobilized MB with its target DNA molecule. [DNA] = 100 nM. All experiments were performed in hybridization buffer of 20 mM Tris-HCl, pH 8.0, with different salts.

### Table 1. Effect of Metal Ions on the Fluorescence Intensity of Molecular Beacons Immobilized on an Optical Fiber Surface

<table>
<thead>
<tr>
<th>Metal Ions</th>
<th>0</th>
<th>10 mM</th>
<th>30 mM</th>
<th>100 mM</th>
<th>300 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>73.80</td>
<td>68.30</td>
<td>17.35</td>
<td>36.22</td>
<td>7.76</td>
</tr>
<tr>
<td>NaCl</td>
<td>68.30</td>
<td>67.00</td>
<td>16.03</td>
<td>36.22</td>
<td>7.76</td>
</tr>
<tr>
<td>MgCl(_2)</td>
<td>28.60</td>
<td>28.60</td>
<td>5.12</td>
<td>28.60</td>
<td>5.12</td>
</tr>
</tbody>
</table>


in MBs before hybridization and the hybrid formed between the MB loop and its target. Therefore, in the presence of divalent ions the hybridization reaction rates are greatly improved. The cations stabilize the base pairs in the following order: Ca\(^{2+}\) > Mg\(^{2+}\) > K\(^{+}\) > Na\(^{+}\), showing that both the charge and the ionic radius affect the stabilization of the newly formed duplex.\(^{25}\) One thing worth noting is that when a double-stranded DNA is short, like the 5 base pairs in the stem of the MB in this work, the influence of K\(^{+}\) and Na\(^{+}\) on the double-stranded DNA stability is not evident.

Most molecular beacon assays done so far have been carried out in the presence of low millimolar range MgCl\(_2\). We investigated the influence of Mg\(^{2+}\) concentration on hybridization kinetics of the immobilized MB with its target DNA on an optical fiber surface. The complementary oligonucleotide concentration was 100 nM. As MgCl\(_2\) concentration was increased, the hybridization rate was greatly improved as shown in Figure 4. The effect of MgCl\(_2\) on the hybridization reaction on the surface is, in essence, independent of the MgCl\(_2\) when the concentration of MgCl\(_2\) is above 100 mM.

**Sensitive Detection of Target DNA Molecules.** We have determined the sensitivity of the MB evanescent wave sensor and further studied the kinetics of the hybridization reaction on the fiber surface by using different concentrations of target oligonucleotide. The target DNA can directly hybridize with the immobilized MB molecules on the fiber surface. Initial reaction rates are used to characterize the hybridization kinetics. The rate is plotted vs concentration of target DNA in a 0–100 nM range, as shown in Figure 5. The hybridization rate is increased linearly with increasing target oligonucleotide concentration. The excellent linear relationship (with a correlation factor of 0.9987) between the initial reaction rate and target DNA concentration indicates that the initial binding kinetics on the optical fiber surface are a pseudo-first-order reaction. We have carried out statistical determination of the detection limit of the evanescent wave MB DNA sensor. We used the slope of the line in Figure 5 to calculate the detection limit. The slope is 0.00126, while the standard deviation (\(\sigma\)) for the baseline obtained before the hybridization started is 0.000462. The concentration detection limit of the sensor (S/N = 3) for a target DNA is 1.1 nM. Using initial reaction rates to characterize the sensor’s detection limit is more appropriate than steady-state fluorescence intensity. The reaction rate is an instant measurement dependent on target DNA concentration under fixed and optimal conditions. When the target DNA concentration is low, it takes a long time to reach steady-state.

**Determination of \(\gamma\)-Actin mRNA Sequences.** Evanescent wave DNA biosensors, using molecular beacon B, have been developed specifically for the determination of rat \(\gamma\)-actin mRNA sequences. The loop sequence of the immobilized MB probe corresponds to the antisense strand, complementary to the rat \(\gamma\)-actin mRNA (bases 815–832) or human \(\gamma\)-actin mRNA (bases 833–850). The MB evanescent wave sensor was applied to detect the 204-nt rat \(\gamma\)-actin mRNA fragment (bases 782–985) amplified by the polymerase chain reaction. The 204-nt mRNA was denatured for 5 min at 95 °C and cooled on ice. The MB biosensor was inserted directly into the 204-nt mRNA buffer solution. The fluorescent intensity was monitored over time. We were able to detect the RNA concentration to as low as 20 nM. The result demonstrates the potential of the biosensor for the quantitation of mRNA and PCR products. The signal generated by the RNA sample was lower than that generated by the complementary oligonucleotide with the same concentration. This suggests that intramolecular RNA folding may compete with the RNA-immobilized MB interaction. It is expected that the MB sensor will be able to determine minute amounts of mRNA with improved detection limit. The detection limit of this sensor can be mainly achieved by new design of the MB.\(^{16}\) Other areas of improvement for the detection limit are a better MB immobilization method on an optical fiber surface, better coupling of the optics, more appropriate hybridization conditions (such as temperature control, etc.).

**DISCUSSION**

The hybridization kinetics are of great interest when studied at a liquid–solid interface. As shown in Figure 3, in the presence of 30 mM monovalent ions the hybridization reaction on the fiber surface was obviously impeded at the early stage, especially for the hybridization reaction in the presence of 30 mM NaCl. The electric repulsion may be the limiting factor which controls the diffusion of target oligonucleotide to the sensor surface at the early stage of the hybridization. To elucidate the mechanisms, we conducted a hybridization experiment in a solution containing a 20 mM MB probe, 100 nM complementary DNA in a 20 mM Tris-HCl buffer, and 30 mM NaCl. We have not observed any delay in hybridization as that shown in Figure 3 (for 30 mM NaCl).
another experiment, we tested the same hybridization reaction on the fiber surface in the presence of 300 mM NaCl (instead of 30 mM). The delay in the hybridization reaction on the surface was not observed either. These two experimental results thus suggest that, when low concentrations of monovalent cations are used, the diffusion of complementary DNA to the sensor surface plays an important role in DNA hybridization kinetics at the early stages.

In most of the kinetics studies of biosensors, the hybridization kinetics do not follow the ideal binding curve for a pseudo-first-order reaction. A variety of interpretations and analysis methods have been developed in the literature to explain this phenomenon. Among them is the idea that the deviations may be due to availability of the biomolecules immobilized on the sensor surface. Most current biosensors are prepared using a matrix, such as dextrin gel, for the immobilization of biomolecules for binding sites at the sensor surface. Mass transport limitations within the dextrin matrix thus play an important role in determining the kinetics of the biosensor. The mass transport limitations are identified as the origin of the deviations from the ideal binding curve. The molecular beacon DNA sensor prepared in this work has no matrix. Therefore, the target DNA molecules have direct access to the immobilized MB molecules on the sensor surface. In the presence of high MgCl$_2$ concentration, the diffusion process may be neglected. The initial binding kinetics of the hybridization reaction on the optical fiber surface is thus observed to be a pseudo-first-order reaction. The oligomer length and steric shape of the oligomer may also have some impact on the MB sensor hybridization reaction rate. These effects have been studied in the literature.

CONCLUSION

Optical fiber evanescent wave molecular beacon DNA sensors have been developed, characterized, and applied in mRNA detection. Molecular beacons are oligonucleotide probes that become fluorescent upon hybridization with target DNA/RNA molecules. The MB based DNA sensor does not require dye-labeled analyte nor intercalation reagents. The signal transduction mechanism is built within the MB molecule, and there is no need to run competitive assays. A biotinylated MB has been designed and synthesized for the development of DNA/RNA biosensors through (strept)avidin–biotin binding on an optical fiber surface. The MB DNA sensor can detect nonlabeled DNA target in real time with high sensitivity and one-base-mismatch selectivity. The concentration detection limit of the MB evanescent wave sensor for its target is 1.1 nM. The sensor is small, rapid, stable, and reproducible. It has remote detection capability. Effects of ionic strength on the hybridization kinetics have been investigated. Divalent ions play more important roles than monovalent ions in stabilizing the stem structure of the MB probe and also in accelerating hybridization reactions. The sensor has been applied for the detection of specific mRNA sequences amplified by the polymerase chain reaction. We expect the MB based DNA biosensors will be useful in diagnostics and in biotechnology.

Using a well-developed technique in detecting single biomolecules and studying their interaction, we expect that better understanding will be achieved on the MB hybridization kinetics. The hybridization kinetics of surface-immobilized MB and MB in free solution will be compared. Important insights into sensor mechanisms and the way to improve sensor’s performance will be obtained. Molecular beacons hold great promise in studies in genetics, disease mechanisms, and disease diagnostics. Efforts are being made to explore their applications in different areas. More research is expected for the mutation detection for a variety of diseases and for RNA monitoring in living cells. This will be expedited with the help of the development of ultrasensitive MB DNA biosensors.

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