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Neutralized chimeric DNA probe for detection of single nucleotide polymorphism on surface plasmon resonance biosensor

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ABSTRACT

An implementation of neutralized chimeric DNA oligomer as a probe for sensitive detection of single nucleotide polymorphisms (SNPs) on a surface plasmon resonance imaging sensor is investigated. The chimeric DNA oligomer was synthesized in a conventional DNA synthesizer, containing neutral nucleotides with a methylated phosphate group. The secondary structures and melting points of the chimeric DNA fragment and its complexes with perfect-matched and single-mismatched complementary DNA molecules were examined by using circular dichroism and UV–vis spectroscopy in comparison with the native probe DNA counterpart. The results indicate that the chimeric DNA complexes can form a B-form structure and exhibit high thermostability. Moreover, the hybridization and discrimination efficiency of the chimeric probe DNA for the SNP genotyping were verified by using the SPRi sensor under different experimental conditions. The data reveal the effects of the ionic strength and operation temperature on the selectivity of the chimeric probe DNA for the SNP detection. The hybridization condition with a low ionic strength and high temperature allows the chimeric probe DNA distinguishing perfect-matched and single-mismatched target DNA molecules to the best extent, likely due to the reduced electrostatic repulsive force and presence of the additional methyl group on the backbone. Consequently, the direct and label-free detection with the SPR technique and neutralized chimeric probe DNA can be realized for the SNP genotyping by optimizing the operation condition and sequence design.

1. Introduction

Single nucleotide polymorphisms (SNPs) are frequently occurring genetic variation in the genome that is found in more than 1% of the population (Kypr et al., 2009). They have become important indicators to link sequence variations to phenotypic changes and to allow biologists in detecting genes related to common diseases. Therefore, over a decade a plenty of efforts have been devoted to developing rapid, sensitive, specific and affordable technologies for SNP analysis, which include differential hybridization strategies, single-base extension after amplification, mismatch endonuclease-based detection and DNA sequencing (Chakrabarti and Schwarz, 1999; Chang et al., 2017; Hur et al., 2015; Petrovykh et al., 2003; Piliarik et al., 2010, 2005; Schwarz et al., 1999; Zhao et al., 1999). However, most of them relay on approaches to amplify sample numbers and detection signals, which may lead to high cost, large noises, cumbersome operation and inefficiency in genotyping. In addition, the readouts from aforementioned techniques are not able to interrogate the kinetic and dynamic information from the target DNA fragments with mutant and wild-type reference DNA. Developing a better understanding of DNA hybridization will greatly facilitate the elucidation of molecular mechanisms involved in numerous biochemical processes and the sophisticated relation with diseases (Islam et al., 2017; Piliarik et al., 2010). Therefore, it is highly desirable to develop a new sensing technology for real-time SNP detections in a sensitive and specific way.

Surface plasmon resonance (SPR) biosensor is a popular optical analytical tool in which an analyzed liquid sample with target molecules is brought in contact with a metal sensor surface (Wu et al., 2017). The metal surface supports resonantly excited surface plasmons by coupling the incident light on the basis of fulfilling the phase matching condition. The capture of analyte molecules by the probe elements immobilized on the surface is sensitively detected upon induced refractive index changes at the interface. The SPR biosensors afford label-free and in-situ molecular detection with very high sensitivity for a wide spectrum of

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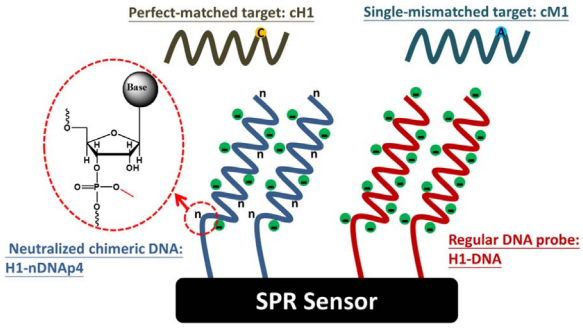
applications, including DNA hybridization events (Homola, 2003; Piliarik and Homola, 2008; Piliarik et al., 2009, 2005; Shushama et al., 2017). Real-time detection of DNA manipulation such as hybridization kinetics, and enzymatic modifications using SPR biosensors was initially reported by Nygren et al. (Nilsson et al., 1995). They demonstrated a methodology to discriminate between probes that are either fully matched to the target sequence or contain single nucleotide mismatches by analyzing hybridization kinetics at different temperatures (Persson et al., 1997). The advances have greatly improved the process for the DNA analysis using traditional approaches and, however, a direct detection for single nucleotide mutation was still required for fast and high-throughput screening. Nakatani et al. introduced synthetic ligands that specifically bind with high affinity to the guanine (G)–guanine mismatch, one of four types of SNP (Nakatani et al., 2001). A series of small specific ligands for targeting mismatched base pairs in DNA duplexes were immobilized on a SPR sensor and used to successfully and directly detect the G-G, G-A and C-C mismatches in hetroduplexes or trinucleotide repeats (Hagihara et al., 2004; Kobori et al., 2004; Nakatani et al., 2001).

Recently, considerable efforts have been contributed to design of DNA analogues with novel properties unfound in nature to improve stability, functionality and binding characteristics. These have been utilized to develop innovative therapeutic agents and new probes for diagnostics. Several non-natural nucleosides have been implemented, such as locked nucleic acid (LNA), peptide nucleic acid (PNA), phosphoramidates morpholino (MORF), and hexitol nucleic acid (HNA) oligomers, which have proven advantages over regular nucleic acids in terms of biostability in body fluids without compromising the specific interaction with complementary target nucleic acids (Briones and Moreno, 2012). PNA is composed of the electrically neutral peptidomimetic backbone, and it retains the ability of forming duplexes with complementary DNA (cDNA) with improved hybridizing affinity. This is attributed to the lack of electrostatic repulsion occurring between DNA strands. In previous works, PNA probes were used for detection of single-mismatched DNA targets with high specificity and sensitivity in adequate buffers and temperatures (Burgener et al., 2000; Lao et al., 2009), and in standard PBS buffer (Ananthanawat et al., 2010). Coordinately, applications of LNA benefit from the conformational restricted sugar motifs and the possibility of synthesizing chimeric molecules, containing both LNA nucleotides and DNA or RNA nucleotides. LNA has been showed also to form the strongest duplexes with complementary RNA, particular microRNA, according to Watson–Crick rules (Obika et al., 1998; Petersen and Wengel, 2003). In addition to PNAs and LNAs, the other uncharged DNA analogues, phosphorodiamidate morpholino oligomers (PMO) and methyl-phosphonate oligonucleotides (MPO), are synthesized for therapeutic applications. The uncharged character of DNA analogues enables to avoid the interferences in a binding reaction from background electric charges in the field-effect transistors (FET) measurements for sensitive DNA detection (Gao et al., 2007). Therefore, on the basis of the current successful examples, the DNA sensors incorporated with artificial probes possess considerable applicability in a growing number of the future analysis of genomics and diagnosis.

We currently described the fully electrically neutral DNA (nDNA) with the “RO-P-O” backbone (wherein R can be methyl, ethyl, aryl, or alkyl group) to evaluate the difference in DNA detection performance on FET and SPR imaging biosensors (Chen et al., 2013). The circular dichroism (CD) spectroscopy analysis indicated that the nDNA holds the capability to specifically hybridize with its cDNA and to form the identical secondary structures as the DNA/cDNA duplex. Furthermore, it was demonstrated that using the nDNA probe in the FET measurement leads to significant improvement in the detection sensitivity due to the electrically neutral property of nDNA to avoid the interference of charge noise from recognition elements and to afford high surface density of probe molecules on sensor chips.

In this work, we demonstrate the feasibility of the chimeric DNA molecule, composed of native and methylated neutral nucleotides, as a probe for sensitive and specific SNP genotyping on label-free real-time SPR imaging biosensor (SPRi) (Scheme 1). The target fragment, cH1, is hemagglutinin 1 DNA in influenza virus strains, which mutates frequently over time and is strongly associated with diverse genetic diseases. The SNP detection for cH1 is critical in infectious disease surveillance and management for reducing the morbidity and mortality of illness. In this study, the methylated nucleotides allow DNA synthesis with native nucleotides in conventional DNA synthesizers to afford ease of implementation in industrial, and versatility in design of sequence and duplex structure. The formation and secondary structure of the duplex of the chimeric DNA probe with target cH1 were verified using CD spectroscopy. The dissociation melting points of the duplexes with and without mismatched point were determined by UV–vis spectrometer with a temperature control module. For the preparation of sensor chips, the chimeric DNA probes were immobilized via aldehyde-amine conjugation chemistry, which was confirmed by X-ray photoelectron spectroscopy (XPS) measurements. Importantly, the SPRi biosensor was employed to investigate the hybridization efficiency of selectively neutralized DNA probe and target cH1. The effects of the ionic strength and hybridization temperature were put into account with a purpose to discriminate from the single base mismatched DNA for potential use in the SNP genotyping. Consequently, given the rise in advanced sensing technologies, we envision that the analytical technology combined with potent biorecognition element and label-free optical sensor could serve as a promising tool in SNP validation in a rapid and sensitive fashion.



Scheme 1. Schematic illustration of probe and target design for SNP detection.

2. Materials and methods

2.1. Chemical reagents

PBS solution contains 10 mM phosphate buffer, 137 mM sodium chloride, and 2.7 mM potassium chloride, adjusted to pH7.4. Orthophosphoric acid, sodium cyanoborohydride, tris(hydroxymethyl) aminomethane (Tris), trimethylamine (TEA), glycerol, 11-amino-1-undecanethiol hydrochloride (MUAM) and 6-mercapto-1-hexanol (MCH) were bought from Sigma-Aldrich (USA). Glutaraldehyde (25%) was purchased from Fluka (USA). Sodium chloride (NaCl), sodium phosphate tribasic, absolute ethanol (99.5%), and ethanolamine (C_2H_7NO) were acquired from Acros Organics (USA). All other chemicals used in this study were reagent grade.

2.2. Regular and neutral sequences

The sequences of regular DNA fragments and their chimeric counterparts were all the same and the difference between these two kinds of DNAs was found in their structure (Table 1). Chimeric DNA was an oligonucleotide analogue and was supplied by Helios Bioelectronics Inc. (Hsinchu, Taiwan) and all oligomers were synthesized by MDBio Inc. (Taiwan). The DNA analogue with the “RO-P-O” backbone (wherein R is methyl group, Scheme 1) used in this study was synthesized by using Fmoc-protected phosphoramidites. The neutralized probe H1-nDNAp4 has four methylated nucleotides in the backbone as noted by the super-