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A comparative study of a label-free DNA capacitive sensor using a pyrrolidinyl peptide nucleic acid probe immobilized through polyphenylenediamine and polytyramine non-conducting polymers

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ABSTRACT

This work has, for the first time, reported a comparative study on the performances of a label-free capacitive DNA sensor based on an immobilized pyrrolidinyl peptide nucleic acid with a D-prolyl-2-aminocyclopentanecarboxylic acid backbone (acpcPNA) attached to three easy to prepare non-conducting polymers. Two phenylenediamine (PD) isomers (*ortho*- and *para*-) and one tyramine monomer were electropolymerized to form polymer films. The hybridization between the acpcPNA probes, immobilized on the polymer layer, and the target DNA was detected directly by measuring the capacitance change. Using the optimal electropolymerized conditions, the highest sensitivity of 20.9 ± 0.6-nF cm^{-2} (log M)$^{-1}$ was obtained from poly-*para*-PD (P*p*PD) with a lowest detection limit of 0.2 pM. A wide linear range, 1.0×10^{-12} to 1.0×10^{-8} M, was obtained for both the P*p*PD and polytyramine (Pty). A narrower linear range of 1.0×10^{-11} to 1.0×10^{-8} M was obtained from the poly-*ortho*-PD (P*o*PD). The surface morphology was observed using scanning electron microscopy and atomic force microscopy. The polymer with the better surface morphology, i.e., smoother, denser and more homogeneous was the one with more immobilized probes and a better overall performance. All modified polymers gave a high % signal suppression (%SS) for both the single and double mismatched target DNAs, and indicated its high specificity. The electrodes can be reused for at least 68 analytical cycles. The effects of the target length and probe concentrations were also investigated. Under the operating conditions of this work the system would be very useful for the cost-effective analysis of ultra-trace levels of DNA in samples.

1. Introduction

Label-free DNA sensors are now being widely investigated due to their low cost, simplicity of operation and fast response times. In recent years, there has been an increase in the use of synthetic peptide nucleic acid (PNA) probes instead of DNA probes because of their higher stability [1]. They also provide a higher specificity with faster hybridization kinetics [2,3]. Many transducers have been successfully employed for label-free DNA detection such as mass sensitive [4,5], optical [6,7] and electrochemical transducers [8,9]. One of the systems that has attracted recent interest due to its high sensitivity, simplicity of operation and is relatively inexpensive is the potential step capacitive technique [10–13].

The principle of the potential step capacitive system is based on measuring changes in dielectric properties when a target binds with the sensing element on the electrode surface. One of the important factors to facilitate detection of the change is the insulating property of the immobilized surface. This is to prevent possible interference from electroactive species in the electrolyte solution and reduces the Faradaic current [14,15]. The insulating layer is generally based on the use of a self-assembled monolayer (SAM) of thiol compounds [9,10,12,14–16] for the immobilization of the sensing elements. However, the self-assembly process requires a relatively long preparation time (12–24 h) and a very smooth surface of the gold electrode is a necessity [16,17]. To overcome these problems electropolymerization of a non-conducting

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polymer on the working electrode is an attractive alternative. It can be produced much more quickly (8 min) and the roughness of the gold electrode is not such a critical factor for the formation of the polymer [18]. Furthermore, these insulator layers [19,20] can be prepared as an ultra-thin film (10–100 nm) [21–24] and provides a large number of surface amine group reactive sites for the immobilization of sensing molecules [19]. Poly-*ortho*-phenylenediamine (P*o*PD) [17,25], poly-*para*-phenylenediamine (P*p*PD) [24], and polytyramine (Pty) [16,26] are non-conducting polymers that have been successfully employed as immobilization films for various recognition elements. However, there has not been any report on a DNA sensor based on an immobilized PNA probe prepared on such polymers.

The objective of this work was to compare the performances of a label-free capacitive DNA sensor using an acpcPNA probe immobilized on each of three electropolymerized non-conducting polymers of P*o*PD, P*p*PD and Pty. This is the first time such a comparative study has been attempted. The optimal electropolymerized conditions, i.e., the monomer concentration and number of scans, were first investigated together for each polymer. This is also the first time the performances were compared under optimal conditions for each polymer. The sensing element was acpcPNA, a conformationally constrained peptide nucleic acid derived from a D-prolyl-2-aminocyclopentanecarboxylic acid (ACPC) backbone. This is a relatively new PNA system developed by our group [27,28]. This PNA provides a stronger binding affinity and better sequence specificity toward DNA compared to DNA itself [29,30] and aminoethylglycyl PNA (aegPNA) [4,28,31]. The surface morphology of the polymer films was observed using scanning electron microscopy and images obtained from an atomic force microscope. These together with the amount of the immobilized acpcPNA probes were correlated to the performances.

2. Experimental

2.1. Materials

The 9-mer lysine-modified acpcPNA (Ac-TTTTTTTTT-LysNH_2) denoted as 'acpcPNA-T9 probe' and the 12-mer mixed base lysine-modified acpcPNA (Bz-TGTCAACTGACT-LysNH_2) denoted as 'acpcPNA-M12 probe', were synthesized by Mrs. Chotima Vilaivan at Chulalongkorn University, Thailand according to the published protocol [27,28]. The acpcPNA probes were purified by reverse phase HPLC (to >90% purity) and their identities were verified by MALDI-TOF mass spectrometry (acpcPNA-T9: *m*/*z* calcd. 3179.4 found 3179.4; acpcPNA-M12: *m*/*z* calcd. 4270.6 found 4271.6).

Oligonucleotides (DNAs), complementary, single mismatched, double mismatched and non-complementary target DNAs were from Bioservice Unit, National Science and Technology Development Agency and BioDesign Co., Ltd., Thailand. The DNA sequences are shown as follows (non-complementary regions are indicated by italicized letters):

For the acpcPNA-T9 probe
Complementary DNA
D6comp: 5′-AAAAAA-3′
D9comp: 5′-AAAAAAAAA-3′
D12comp: 5′-AAAAAAAAAAAA-3′
D27comp: 5′-AAAAAAAAAAAAAAAAAAAAAAAAAAA-3′
D27compM18a: 5′-*TTTTTTTTT*AAAAAAAAA*TTTTTTTTT*-3′
D27compM18b: 5′-*TGCTCAGCT*AAAAAAAAA*TGCTCAGCT*-3′
Single mismatched DNA
D9M1G: 5′-AAAA*G*AAAA-3′
D9M1C: 5′-AAAA*C*AAAA-3′
D9M1T: 5′-AAAA*T*AAAA-3′
Double mismatched DNA
D9M2G: 5′-AAA*G*A*G*AAA-3′
D9M2C: 5′-AAA*C*A*C*AAA-3′
D9M2T: 5′-AAA*T*A*T*AAA-3′
Non-complementary DNA
D9non-compa: 5′-*TTTTTTTTT*-3′
D9non-compb: 5′-*TATTATTAT*-3′

For the acpcPNA-M12 probe
Complementary DNA
D9comp: 5′-CAGTTGACA-3′
D12comp: 5′-AGTCAGTTGACA-3′
D15compM3: 5′-*GCT*AGTCAGTTGACA-3′
D30compM18: 5′-*GACTGCACC*AGTCAGTTGACA*TGTTGCGAC*-3′
Single mismatched DNA
D12M1T: 5′-AGTCA*T*TTGACA-3′
D12M1C: 5′-AGTCA*C*TTGACA-3′
D12M1A: 5′-AGTCA*A*TTGACA-3′
Double mismatched DNA
D12M2T: 5′-AG*TT*A*T*TTGACA-3′
D12M2CG: 5′-AGTCAGTTGACA-3′
D12M2A: 5′-AG*TA*A*T*TGACA-3′
Non-complementary DNA
D12non-comp: 5′-*TCAGTCAACTGT*-3′

The three non-conducting monomers, *ortho*-phenylenediamine (*o*-PD), *para*-phenylenediamine (*p*-PD) and tyramine were obtained from BDH (Poole, England), laboratory UNILAB reagent (Sydney–Melbourne, Australia) and Aldrich (Steinheim, Germany), respectively. Glutaraldehyde and 11-mercapto-1-undecanol (11-MUL) were obtained from Sigma-Aldrich® (Steinheim, Germany). All buffers were prepared with deionized water treated with a reverse osmosis-deionizing system (Pentair, Inc., USA). Before use, buffers were filtered through a nylon membrane filter (Vertical®, Albet, Spain, pore size 0.2 μm) with subsequent degassing. Other chemicals were of analytical grade and were used as received.

2.2. Gold electrode preparation

Gold rod electrodes (99.99% purity) with a diameter of 3.0 mm were polished by hand using alumina slurries with particle diameters of 5, 1 and 0.3 μm, respectively and subsequently cleaned by rinsing with distilled water. The electrodes were then placed in a plasma cleaner (Model PDC-32G, Harrick, New York, USA) for 15 min to remove any organic materials adsorbed onto the surface electrode.

2.3. acpcPNA probe immobilization

The acpcPNA-T9 probe possessing a T9 sequence was chosen for the initial optimization of the model because it is the simplest probe that can form a sufficiently stable hybrid with a complementary DNA target at room temperature ($T_m \sim 73\,^{\circ}C$) and this probe forms only a duplex (not a triplex) with its complementary DNA (dA_9) [28]. Because of its short length and simple composition, this PNA can be synthesized with high efficiency. Furthermore the homo-T sequence contains no exocyclic amino groups that could complicate the immobilization and it also has no electroactive G base that may complicate the interpretation of the results [27,28].

Monomers of *o*-PD and *p*-PD were prepared in 10 mM sodium acetate buffer pH 5.18 [17] and the tyramine monomer was prepared in a solution containing 2.0 mM phosphate buffer pH 7.00 and ethanol (volume ratio of 3:1) [16]. For the immobilization of acpcPNA-T9, the monomer concentration and the number of scans for electropolymerization were optimized at the same time. From the literature 5 mM of *o*-PD [21,32,33], 5 mM of *p*-PD [21,32], and 50 mM of tyramine [16,26] have been used. Therefore, monomer concentrations close to these values were used. For *o*-PD and *p*-PD, 1, 5, 10 and 20 mM of monomer was electropolymerized by cyclic voltammetry at 5, 10, 15 and 20 scans using the potential range from 0.0 to 0.8 V vs. Ag/AgCl with a scan rate of 50 mV s^{-1}. Electropolymerization of tyramine was performed using the same conditions but at concentrations of 5, 15, 35, 50 and 60 mM. The