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Immobilization-free electrochemical DNA detection with anthraquinone-labeled pyrrolidinyl peptide nucleic acid probe

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ABSTRACT

Electrochemical detection provides a simple, rapid, sensitive and inexpensive method for DNA detection. In traditional electrochemical DNA biosensors, the probe is immobilized onto the electrode. Hybridization with the DNA target causes a change in electrochemical signal, either from the intrinsic signal of the probe/target or through a label or a redox indicator. The major drawback of this approach is the requirement for probe immobilization in a controlled fashion. In this research, we take the advantage of different electrostatic properties between PNA and DNA to develop an immobilization-free approach for highly sequence-specific electrochemical DNA sensing on a screen-printed carbon electrode (SPCE) using a square-wave voltammetric (SWV) technique. Anthraquinone-labeled pyrrolidinyl peptide nucleic acid (AQ-PNA) was employed as a probe together with an SPCE that was modified with a positively-charged polymer (poly quaternized-(dimethylamino-ethyl)methacrylate, PQDMAEMA). The electrostatic attraction between the negatively-charged PNA–DNA duplex and the positively-charged modified SPCE attributes to the higher signal of PNA–DNA duplex than that of the electrostatically neutral PNA probe, resulting in a signal change. The calibration curve of this proposed method exhibited a linear range between 0.35 and 50 nM of DNA target with a limit of detection of 0.13 nM ($3SD_{blank}$/Slope). The sub-nanomolar detection limit together with a small sample volume required (20 μL) allowed detection of <10 fmol (<1 ng) of DNA. With the high specificity of the pyrrolidinyl PNA probe used, excellent discrimination between complementary and various single-mismatched DNA targets was obtained. An application of this new platform for a sensitive and specific detection of isothermally-amplified shrimp's white spot syndrome virus (WSSV) DNA was successfully demonstrated.

1. Introduction

The ability to precisely and sensitively identify specific DNA sequence has found widespread applications in many areas e.g. medical diagnostics, agricultural and food sciences [1], and environmental monitoring [2]. In addition to the direct sequencing that require expensive instrument and gel-based approaches that are labor intensive and time-consuming, various DNA sensing platforms that are more suitable for point-of-care diagnostics have been developed over the past decades [3–5]. Among these, electrochemical DNA biosensors offer several advantages including the operational simplicity, high sensitivity, miniaturization and low cost of instrument [6,7]. In general, a probe that can specifically recognize the nucleic acid target is first immobilized onto the electrode. Binding to the target results in a measurable signal change either directly or through a redox-active indicator or labels [8–14]. The most frequently employed immobilization methods include gold–thiol interaction [15–17] and amide bond formation [18,19]. The immobilization step is time-consuming, requires large excess of the probe, and needs careful optimization. Attempts were made to avoid this by using biotin-streptavidin [20,21], host–guest interaction [22], or by electrostatic adsorption [23]. Alternatively, an intercalating redox indicator [24] or a redox-active probe [25–27] had been used in combination with PCR or other

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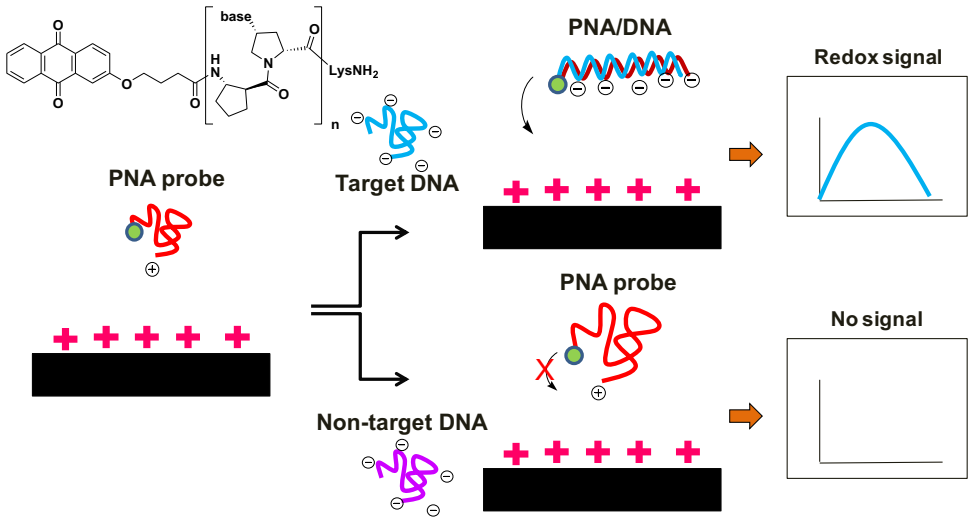

Fig. 1. The principle of immobilization-free electrochemical DNA biosensor employing AQ-PNA probe (structure of AQ-PNA shown in the inset).

enzymatic reactions for immobilization-free DNA detection.

Although DNA had been the most frequently employed probe, several new DNA analogs such as locked nucleic acid (LNA), morpholino and peptide nucleic acid (PNA) are becoming more popular alternatives because they can offer additional advantages including higher sensitivity, specificity and stability [28]. We recently reported a new conformationally constrained pyrrolidinyl peptide nucleic acid that showed excellent DNA hybridization properties [29–31] and demonstrated its applications as a probe for DNA sensing [32,33], including electrochemical detection [34–36].

The electrostatically neutral backbone of PNA allows designing of novel immobilization/detection concepts [37,38]. Luo et al. had recently developed an immobilization-free DNA sensing platform employing ferrocene-labeled PNA probes and indium-tin oxide (ITO) electrodes [39–41]. They observed that the signal detection can be switched from on-off to off-on mode by modifying the surface charge of the electrode [39]. Here we propose an immobilization-free electrochemical DNA sensing platform employing PNA and a polymer-modified screen-printed carbon electrode (SPCE), which can be easily and inexpensively prepared in one step. The electrostatic properties of the electrode can be readily controlled by adding the polymer modifier into the carbon ink without the need to form a monolayer or coating as usually the cases with other electrodes. A practical application was demonstrated in a highly specific detection of WSSV-a shrimp viral pathogen that causes large economic losses to shrimp aquaculture [42].

2. Material and methods

2.1. General materials and methods

Graphite powder (mesh size < 100) was purchased from Sigma-Aldrich. Carbon ink and silver/silver chloride were purchased from Acheson (California, USA). Other reagents and solvents were obtained from commercial suppliers and used without further purification. Poly quaternized-(dimethylamino)ethyl methacrylate (PQDMAEMA, $M_n = 1.38 \times 10^4$ Da) was synthesized and supplied by Dr. Voravee Hoven and Miss Pornpen Sae-ung (Chulalongkorn University). NMR spectra were recorded on a Bruker Avance 400 instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts were referenced to the residual protonated solvent peaks. MALDI-TOF MS spectra were measured on a Microflex MALDI-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) in linear positive mode using α-cyano-4-hydroxycinnamic acid (CCA) matrix. Synthetic oligodeoxynucleotides were purchased from Pacific Science (Bangkok, Thailand) and were used as received. The sequences of PNA and DNA oligonucleotides used in this study, which correspond to a partial sequence of WSSV, are as follows:

AQ-labeled WSSV PNA probe (AQ-PNA): 2AQ-CTAAATTCAGA-LysNH$_2$

Target DNA (Dcomp): 5′-TCTGAATTTAG-3′

Single base mismatched DNA (DsmC): 5′-TCTGACTTTAG-3′

Long single stranded complementary DNA (ssDcomp19):
5′-CTAAGTCTGAATTTAGGGG-3′

Long single stranded mismatched DNA (ssDsmC19):
5′-CTAAGTCTGACTTTAGGGG-3′

Long double stranded complementary DNA (dsDcomp19):
5′-CTAAGTCTGAATTTAGGGG-3′
3′-GATTCAGACTTAAATCCCC-5′

Long double stranded mismatched DNA (dsDsmC19):
5′-CTAAGTCTGACTTTAGGGG-3′
3′-GATTCAGACTGAAATCCCC-5′

Other oligonucleotides used in the specificity test include (mismatch positions underlined):

DsmC6: 5′-TCTGACTTTAG-3′ DsmA4: 5′-TCTAAATTTAG-3′
DsmG6: 5′-TCTGAGTTTAG-3′ DsmT4: 5′-TCTTAATTTAG-3′
DsmT6: 5′-TCTGATTTTAG-3′ DsmA7: 5′-TCTGAAATTAG-3′
DsmC5: 5′-TCTGCATTTAG-3′ DsmC7: 5′-TCTGAACTTAG-3′
DsmG5: 5′-TCTGGATTTAG-3′ DsmG7: 5′-TCTGAAGTTAG-3′
DsmT5: 5′-TCTGTATTTAG-3′ DsmA11: 5′-TCTGAATTTAA-3′

2.2. General procedure for synthesis and labeling of the PNA probe

The WSSV PNA probe (AQ-PNA) used in this study is a pyrrolidinyl PNA with prolyl-2-aminocyclopentanecarboxylic acid backbone (acpcPNA) with a sequence of 2AQ-CTAAATTCAGA-LysNH$_2$ (Fig. 1). The unlabeled PNA probe was synthesized by Fmoc solid-phase peptide synthesis on a TentaGel resin with a Rink amide linker following a previously described protocol [30]. The Fmoc group at the *N*-terminus of the PNA on the solid support was next removed by treatment with 2% 1,8-diazabicycloundec-7-ene (DBU) and 20% piperidine in dimethylformamide (DMF) (100 μL) for 5 min. The free PNA (0.5 μmol) was next reacted with 4-(anthraquinone-2-oxy)butyric acid (pre-activated as a pentafluorophenyl ester, 7.5 μmol) and diisopropylethylamine (DIEA) (20 μL) in DMF (100 μL). After completion of the reaction