CHAPTER I

INTRODUCTION

1.1 Introduction

The detection and quantification of specific nucleic acid sequence is very important for a wide range of applications including medical diagnostics, environmental monitoring, forensics sciences and food safety [1].

Many platforms for DNA sequence detection rely on the specific binding between a probe and the DNA target. In addition to natural nucleic acids (DNA, RNA) several kinds of DNA analogues have been developed and employed in DNA sequence detection [2]. Among several types of nucleic acid analogues available to date, peptide nucleic acid (PNA) is one of the most potential candidates as a probe [3]. PNA is a DNA analogue that carries a polyamide instead of the usual sugar phosphate backbone [4]. Its neutral backbone contributes to the excellent stability of PNA-DNA hybrids because of the absence of electrostatic repulsion between the negatively charged phosphate groups [5]. Despite the high affinity, PNA hybridizes to DNA with higher specificity than DNA itself. Moreover, PNA is more stable both chemically and biologically than DNA and RNA. Accordingly, several new PNA had been developed to date [6-8]. Recently, Vilaivan and coworkers introduced a new pyrrolidinyl PNA system with a conformationally-restricted backbone derived from D-proline-2-aminocyclopentanecarboxylic acid (also known as acpcPNA) [9, 10]. This acpcPNA system exhibits higher affinity and specificity to complementary DNA targets than the original PNA system [11]. These properties make acpcPNA as a potential candidate as a probe for DNA sequence detection.

To detect the DNA sequence, the binding event between the probe and the DNA target must be transduction mechanism to yield a measurable signal. Several detection techniques have been employed for DNA detection, examples of which include fluorescence [12-14], surface plasmon resonance [15] and MALDI-TOF mass spectrometry [16]. Among these detection techniques, electrochemical detection is highly attractive because of its high sensitivity, simplicity and low cost of equipment [17, 18].

In electrochemical DNA detection that employs a probe, the probe is usually attached onto the electrode by means of chemical (formation of covalent bonds) or physical methods (adsorption). The first method provide high sensitivity and reusable

electrode, but the preparation of the electrode is time-consuming. As an example, Liao and Xiao [19] used PNA probe immobilized on gold electrodes via a thiol-Au interaction in a DNA detection platform that can distinguish between fully-complementary and mismatched DNA.

The electrode preparation is not required in the other "immobilization-free" method, although the sensitivity and specificity may not be as good [20]. The principle of immobilization-free is based on physical adsorption of the probe on the electrode such as electrostatic or hydrophobic interactions. As an example, Luo and coworkers [21, 22] developed a PNA-based DNA detection system that did not require probe immobilization. Positively-charged polymer-modified indium tin oxide (ITO) electrode was employed in that work.

From the above literature examples, electrochemical DNA detection generally requires expensive electrodes and complicated instrument set up. Screen-printed carbon paste electrode (SPCE) is an alternative electrode that is easy to make, requires non-toxic chemical and low cost of equipment [23]. Chailapakul and coworkers had extensively used this type of electrode for electrochemical detection of heavy metals or organic compounds with excellent results [24, 25]. Recently, Jampasa and coworkers [26] employed immobilized acpcPNA probe on a chitosan-modified SPCE for electrochemical DNA detection of HPV type 16 DNA.

In addition to the probe, a redox-active reporter (which can be integrated into the probe itself or subsequently added following the hybridization between the probe and the target as an "indicator") is an important component in electrochemical DNA biosensors. Many electrochemically active reporters have been used as electron transducer in electrochemical DNA detection [27-31]. Metal-containing species such as $Ir(bpy)(phen)(phi)^{3+}$, $Ru(NH_3)_5Cl^{2+}$, $Rh(phi)_2(dmp)^{3+}$ and $Fe(CN)_6^{4-}$ [28, 30] showed good electron transfer behaviors but their stability is usually not good, especially in aqueous solution. In this respect, organic redox-active reporters such as anthraquinone (AQ) [17, 26, 30, 32, 33], methylene blue (MB) [33-36] nile blue (NB) [37, 38] or riboflavin (RB) [39, 40] are more attractive. In this work, several organic redox-active reporters were linked to acpcPNA via amide bonds to give redox-active acpcPNA probes. Next, an immobilization-free platform for DNA sequence detection employing the redox-active acpcPNA probe and SPCE was designed, optimized and evaluated. Applications of this new platform in the areas of agricultural and medical diagnosis were also demonstrated. For agricultural applications, the detection of White Spot Syndrome Virus (WSSV) infection in shrimps, which is a serious problem in aquaculture and resulted in large economic losses [41],

was selected as the showcase. For medical diagnosis applications, detection of human papilomavirus type 16, which is the cause of cervical cancer [42], and identification of human leukocyte antigen (HLA) B*1502 that is known to associate with some drug hypersensitivities [43] were chosen.

1.2 Objectives of the research

The main objectives of this work are as follow:

1. To synthesize pyrrolidinyl peptide nucleic acid (acpcPNA) labeled with a redox-active reporter
2. To evaluate the hybridization properties of the redox-active acpcPNA
3. To develop an immobilization-free electrochemical platform for DNA detection employing redox-active acpcPNA probes and screen-printed carbon paste electrodes (SPCE)
4. To apply the redox-active acpcPNA-based electrochemical DNA detection platform for detection of DNA samples

1.3 Scope of the research

The pyrrolidinyl peptide nucleic acid (acpcPNA) will be synthesized and labeled with various organic redox-active reporters at terminal and internal positions. The hybridization property of the redox-labeled PNA with complementary and single base mismatched DNA will be evaluated by thermal denaturation experiments. The electrochemical signals of differently labeled acpcPNA will be measured on modified SPCE in the absence and presence of complementary DNA targets. The redox-active PNA probe that provide the best signal strength and shape will be selected for further optimization of various parameters in order to maximize difference between the signals of free and hybridized probes. The linearity range, limit of detection and limit of quantitation will next be determined. Finally, applications in detection of amplified real DNA samples will be demonstrated.