CHAPTER V
CONCLUSION

A novel immobilization-free electrochemical platform for DNA sequence detection by using redox-active acpcPNA as a probe was proposed. Anthraquinone was employed as a redox-active label, which was attached to the *N*-terminal position of acpcPNA via amide bond. The AQ-labeled acpcPNA exhibited increased affinity with complementary DNA compared to unlabled acpcPNA as shown by melting temperature (T_m) measurement, which could be explained by π-π interaction between the label and the terminal base pair. Moreover, the specificity of AQ-labeled acpcPNA was good as shown by a large decrease in thermal stability of mismatched PNA-DNA duplexes. The use of AQ-labeled acpcPNA probes for developing an immobilization-free electrochemical platform for DNA sequence detection on screen printed carbon electrode (SPCE) was next investigated. The detection principle relies on the differential electrostatic interactions between the positively charged lysine-modified labeled acpcPNA probe and negatively charged DNA/PNA-DNA hybrids on a polymer-modified SPCE. Single-stranded AQ-labeled lysine-modified acpcPNA probes gave no signal by square wave volammetry (SWV) on PQDMAEMA-modified SPCE because both the PNA and the electrode carried positive charges. Upon hybridization with the complementary DNA target, the negatively charged PNA-DNA hybrid electrostatically adsorbed onto the positively charged electrode and produced a signal. The parameters were optimized including buffer, amounts of polymers and SWV parameters. Under optimized conditions (10 mM Tris-HCl buffer pH 8.0, 0.26% PQDMAEMA modified SPCE, SWV parameters: frequency 40 Hz, step potential 0.075 V, amplitude 0.1 V and accumulation potential 0.7 V), a linear response was obtained between 10-50 nM DNA. The limit of detection of at least 1 nM and limit of quantitation of 15.8 nM was obtained with 2AQ-WSSV-Lys acpcPNA probe at 50 nM. The detection platform exhibited excellent specificity. The signal was observed only in the presence of complementary DNA. No signal could be detected with various single-mismatched DNA targets even when it was present 1,000 times excess over the complementary DNA. However, large excess of both complementary and non-complementary DNAs over the PNA probe decreased the sensitivity by competitively binding to the electrode. Double-stranded DNA targets required denaturation prior to the hybridization and electrochemical

measurement. Furthermore, applications of this platform for detection of real DNA samples of economical (WSSV) or clinical (HPV, HLA) importance were successfully demonstrated. Finally, the mechanism of the signal change proposed above was confirmed by comparing the SWV signals of acpcPNA bearing different amino acid modifiers (Lys: positive, Ser: neutral; Glu: negative) and their DNA hybrids on different polymer-modified electrodes (PQDMAEMA: positive, none: neutral; PAA: negative)