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Electrochemical DNA Biosensor For Detection Of Porcine Oligonucleotides Using $[Ru(bpy)_2PIP]^{2+}$ Complex

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***Abstract*—An electrochemical DNA biosensor for detection of porcine oligonucleotides based on ruthenium (II) complexruthenium(II) complex, $[Ru(bpy)_2(PIP)]^{2+}$, (bpy = 2,2'bipyridine, PIP = 2-phenylimidazo[4,5-f[[1,10-phenanthroline]) as label redox have been developed. The study was carried out by immobilization of porcine aminated DNA probes sequences on screen printed electrode (SPE) modified with succinimide-acrylic microspheres and $[Ru(bpy)_2(PIP)]^{2+}$ to detect DNA hybridization event. The electrochemical detection by redox active ruthenium (II) complex was measured by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results indicate that the interaction of $[Ru(bpy)_2(PIP)]^{2+}$ with hybridization complementary DNA has higher response compared to single-stranded and mismatch complementary DNA. Under optimized condition, this porcine DNA biosensor shows linear response range towards target DNA within range of 1 x10 $^{-5}$ uM to 1x10^{-13} uM.**

***Keywords*—DNA biosensor, ruthenium, electrochemical**

I. Introduction

DNA (deoxyribonucleic acid) is the most important genetic material that carry the useful information and act as basis of gene expression. More recently, the detection of DNA have tremendous interest for researchers as it plays an important role in many applications such as clinical diagnostic, forensic analysis, food analysis and environmental monitoring [1]. DNA biosensors are integrated receptor-transducer devices that used DNA as recognition element to measure specific binding process with DNA either by thermal, electrical or optical signal transduction [2].Currently, electrochemical DNA biosensor have great demand for DNA sequences analysis [3]. This is due to advantages of electrochemical transduction which are low cost, good sensitivity, simplicity and accurate specificity.

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This is due to advantages of electrochemical transduction which are low cost, good sensitivity, simplicity and accurate specificity. Furthermore, electrochemistry provides ability to control DNA hybridization and denaturing process [4]. In the past few decades, the studies of DNA interaction with metal polypyridyl complex of some transition elements like ruthenium(II), cobalt(II), copper(II) and zinc(II) have been extensively explored to develop biomedical reagent and electrochemical DNA probes [5].Nowadays, ruthenium complexes have gain popularity as redox indicators in variety of biosensor application[6].Ruthenium (II) complexes have good stability for the interaction with DNA in biosensor as its gives stability in term of chemistry, electrochemistry and photophysical behavior towards DNA. Comes together with metal complex, most label based electrochemical DNA biosensors use rigid bidentate ligands, such as 1,10-phenanthroline (phen) or 2,2'-bipyridine (bipy). Both ligands interact differently with single stranded DNA (ssDNA) and double stranded DNA (dsDNA) [7]. The studies found that intercalating ability of metal complexes and DNA was increase with improvement of planarity of ligands.

In this study, we have examined the used of ruthenium (II) complex, $[Ru(bpy)_2(PIP)]^{2+}$ (bpy = 2,2'bipyridine, PIP = 2-phenylimidazo[4,5-f[[1,10-phenanthroline])**(FIGURE 1)** as electrochemical DNA indicator. Selection of this complex is due to the aromatic bipyridine(bpy) ligand as intercalator which have ability to insert itself between the double helix DNA. In addition, according to literature [8], the binding constant, K_b, of this redox indicator is strong which is 4.7 x $10^{\wedge 5}$ M^{-1} . The interaction of DNA and $[Ru(bpy)_2(PIP)]^{2+}$ was studied by electrochemical transduction. For modification of electrode, gold nanoparticles (AuNPs) and succinimide-acrylic microspheres were used to make covalent immobilization with probe ssDNA porcine sequences. The $[Ru(bpy)_2(PIP)$ was used as intercalator during hybridization for detection and interaction of porcine DNA sequences.

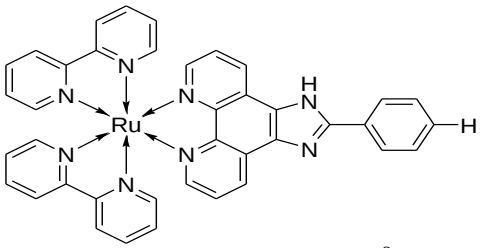


FIGURE 1.$[Ru(bpy)_2(PIP)]^{2+}$

II. Materials and Method

All the electrochemical measurements were obtained using Autolab potentiostat incorporated with General Purpose Electrochemical system (GPES) software. The electrochemical system consists of carbon screen printed electrode (SPE) as working electrode, a carbon pencil as counter electrode and Ag/AgCl as reference electrode. The metal complex, $[Ru(bpy)_2(PIP)]^{2+}$ and succinimide acrylic mircropshere were synthesized according to the literature [9] [10]. All the stock solutions were prepared in postassium phosphate buffer (KPBS). The synthetic of porcine oligonucleotides sequences 5'- CTG ATA GTA GAT TTG TGA TGA CCG TAG[AmC3] (probe), its complimentary strand 5'- CTA CGG TCA TCA CAA ATC TAC TAT CAG-3' were purchased from Sigma Aldrich. Gold nanoparticle was carefully dropped on the screen printed electrode (SPE) and followed by immobilization of succinamide acrylic microsphere. On those modified electrode, probe DNA have been immobilized as the covalent bond was formed at the terminal-NH in the DNA sequences. Hybridization was carried out by immersing modified electrode with probe DNA into the solution containing target porcine DNA sequences, 2M NaCl as supporting electrolyte and 20uM of $[Ru(bpy)_2(PIP)]^{2+}$ as intercalator. Voltammetric transduction measurements used for this experiment were cyclic voltammetry and differential pulse voltammetry.

III. Results and Discussion

A. *Behaviour of $[Ru(bpy)_2PIP]^{2+}$towards DNA interaction*

To explore the application of $[Ru(bpy)_2(PIP)]^{2+}$ in electrochemical DNA biosensor, an electrochemical study of 1.0mM $[Ru(bpy)_2(PIP)]^{2+}$ in 0.05M potassium phosphate buffer (KPBS) pH 7.0 was performed in room temperature. Typical cyclic voltammogram experiment of $[Ru(bpy)_2(PIP)]^{2+}$ in free solution was showed in **FIGURE 2**. There was a reversible redox peak in the range of 0.9 V to 1.2 V. The anodic peak (Epa), which when the ruthenium (II) was oxidized to ruthenium (III) and cathodic peak (Epc), which the ruthenium (III) was reduced back to ruthenium (II), were found at 1.0 V and 1.1 V respectively. The range was accordance to the literature [11] where the $Ru(bpy)_3^{2+}$ peak potential was active. The shifted of peak potential may be due to the ligand interaction of the metal complex.

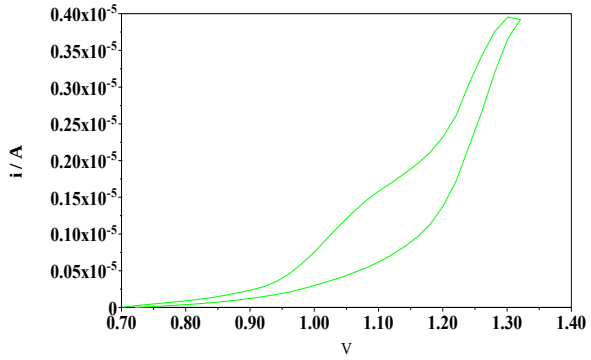
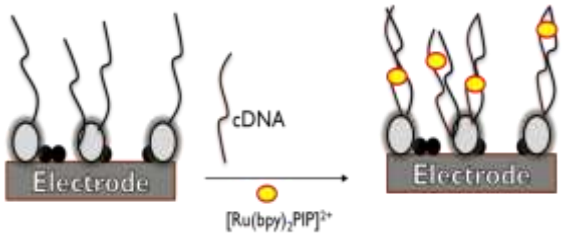
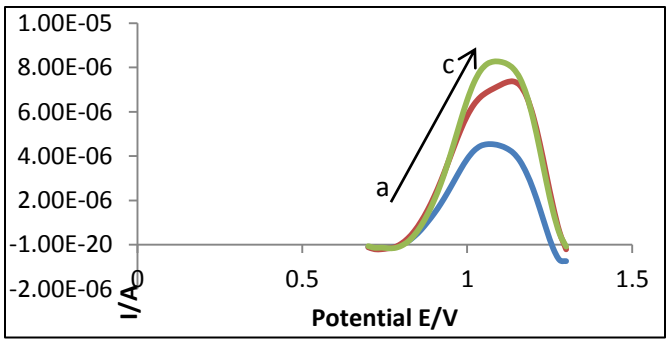


FIGURE 2. Cyclic voltammogram of 1.0mM $[Ru(bpy)_2(PIP)]^{2+}$ in 0.05M potassium phosphate buffer solution (KPBS) pH 7.0 at scan rate 0.02V/s



(A)



(B)

FIGURE 3. (A) Schematic representation of the DNA hybridization detection method using $[Ru(bpy)_2(PIP)]^{2+}$as intercalator label redox on modified screen printed electrode (SPE) (B) Differential pulse voltammogram for interaction of 50uM of $[Ru(bpy)_2(PIP)]^{2+}$ with (a) electrode-modified probe porcine oligonucleotide, (b) after hybridization with mismatch porcine DNA and (c) after hybridization of complementary target porcine DNA sequences

The interaction between $[Ru(bpy)_2(PIP)]^{2+}$ with porcine DNA sequences was also measured by differential pulse voltammetry (DPV) (**FIGURE 3**). DNA hybridization detection strategy is illustrated in **FIGURE 3(A). FIGURE 3(B)** presents the differential pulse voltammogram for interaction of $[Ru(bpy)2(PIP)]^{2+}$ with (a) electrode-modified probe porcine oligonucleotide, (b) after hybridization with mismatch porcine DNA and (c) after hybridization of

complementary target porcine DNA sequences. Significant increase in voltammetric peaks were observed at label redox intercalator, $[Ru(bpy)_2(PIP)]^{2+}$ showing that this label interacts with dsDNA are prominent compared to ssDNA and mismatch DNA sequences. According to the literature [12], most of the interactions between ruthenium (II) polypyridyl complexes are through intercalative mode. Thus, in this experiment, the intensity of the peak was increased as the intercalator inserts itself between the base pairs in dsDNA.

B. *DNA hybridization response*

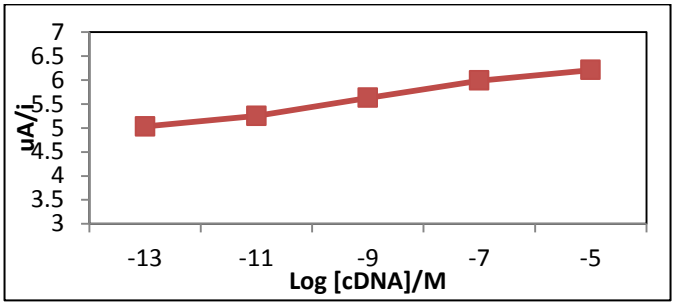


FIGURE 4. The linear range of porcine DNA biosensor response which the hybridization was performed at the optimum condition of 0.05M Na-phosphate buffer, 1.5 M ionic strength for 60 minutes of hybridization time.

FIGURE 4 demonstrates the effect of different concentration of complementary porcine DNA towards biosensor hybridization response. Higher rate of hybridization of DNA occurred by increasing a concentration of the complementary DNA. This current response was linear towards DNA concentration within the range between 1 $x10^{-5}$M to $1x10^{-13}$M. The selectivity, reproducibility, regeneration and life time studies will be conducted to enhance the response of DNA biosensor and give good limit of detection (LOD).

IV. Conclusion

In this preliminary work, interaction of $[Ru(bpy)_2(PIP)]^{2+}$ as intercalator label redox and porcine DNA sequences have been studied. By using electrochemical transduction, cyclic voltammetry (CV) and differential pulse voltammetry (DPV), the interaction between the ruthenium complex as label redox intercalator with single-stranded DNA (ssDNA) and double stranded DNA (dsDNA) can be distinguished. As its role as intercalator, the result indicate that the ruthenium complex have good interaction when the hybridization of complementary target DNA takes place. The parameters of this biosensor work such as concentration DNA, pH, duration of DNA probe immobilization and hybridization, buffer concentrations and ionic strength will be optimized to improve the performance of biosensor.

V. Acknowledgement

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