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Detection of DNA by using bio-conducting polymer–Nile blue composite electrode; Nile blue as an indicator

Zhi-Wei Chen, A. Balamurugan, Shen-Ming Chen *

Electroanalysis and Bioelectrochemistry lab, Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road Taipei 106, Taiwan, ROC

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ABSTRACT

The amplified electrochemical sensing of DNA was accomplished by electrodeposited PEDOT on the electrode surface and incorporation of Nile blue (NB) as redox active intercalator into DNA. Herein, above modified electrode called as PEDOT/DNA/NB composite electrode. PEDOT/DNA/NB composite electrode exhibited well defined redox peak at −0.35 V (Ag/AgCl) corresponding to NB. The composite electrode surface coverage (Γ) and ΔEp were compared with PEDOT/NB and DNA/NB modified electrode. Atomic Force microscopy (AFM), and cyclic voltammetry (CV) were used to characterize the PEDOT/DNA/NB composite electrode. The composite electrode was exhibited as surface confined redox process in neutral pH. The composite electrode was found to be pH dependent. The composite electrode exhibited catalytic property towards reduction of hydrogen peroxide (H_2O_2). The composite electrode was utilized to amperometric study and its response towards H_2O_2 detection was less than 6 s and the detection limit was 0.1 μM. Moreover, we tested PEDOT/DNA/NB composite electrode to electrocatalytic reduction of cytochrome *c* (Cyt *c*).

1. Introduction

The electrostatic and topographic property of biological macromolecules such as DNA complexes can be exploited for the templated generation and assembly of supramolecular aggregates of organic and inorganic building blocks [1,2]. The power of DNA as a molecular template is enhanced by our ability to synthesize virtually any DNA sequence by automated methods, and to amplify any DNA sequence from microscopic to macroscopic quantities by means of polymerase chain reaction (PCR). Therefore, DNA is particularly suitable to serve as a construction component in nano sciences [3]. The use of DNA as template for spontaneous assemblies of cationic cyanine dye [4], fullerene derivatives [5], CdS semiconductor nanoparticles [6], silver nanowires [7], and gold nanowires [8–10] to form supramolecular structures has been extensively reported. In all cases, the negatively charged phosphate backbone of the DNA double helix has been employed to interact electrostatically with inversely charged species presented in solution. Because metal nanoparticles are always wrapped by charged organic layer, the metal nanowires formed by such metal nanoparticles for electronic interconnections of nanometer-scale electronics devices should be resistive [7]. The conductive polymers may provide another option for this problem.

Conductive polymers (CPs) have been extensively studied because of their highly electrical conductivity and mechanical flexibility, their ability to be electrochemically switched between electronically insulating and conducting states. It has been well established that disorder is one of the limiting factors in electron/hole transport in doped conducting polymers [11] and the incorporation of the DNA and probe oligonucleotide into a conducting polymer is attractive because of electrochemical and an electronic properties of conjugated polymers are a sensitive function of their environment, so that hybridization of the incorporated probes would be expected to perturb the electrochemical response of the polymer [12–21]. Also, DNA has a unique secondary structure where a stack of π electrons in the base pair promoted the possibility of DNA to form a "molecular conduit" [22]. Wrapping of a conducting polymer on the DNA surface might be useful to connect the molecular wires [23] and these hybrids might play an important role to bridge the human–machine interface.

Among the conducting polymers, CPs, poly(3,4-ethylenedioxythiophene), or PEDOT, has been reported to exhibit good stability after incorporation of the biological material and to get enhanced electrical signal [24–25]. Since, PEDOT modified conducting polymer films have a high regularity of the polymeric chain due to the lack of α–β linkages between the monomers, a high stability of the p-doped state and a high conductivity [24–26].

Recently, Fang et al. [27] used ferrocene functionalised polythiophene as transducer for lable free DNA detection. Shinkai and co workers [28] prepared PEDOT/DNA/teracationic porphyrin (TMpyP) composite and concluded that DNA is useful as a scaffold to arrange redox active couples in one dimensional matrix. Mouffouk and Higgins [29] reported selective electrochemical response to hybridization based on oligonucleotide-functionalised PEDOT coated micro electrodes. Moreover, Goto et al. [30] prepared PEDOT polymer by using DNA as a liquid crystal electrolyte and the prepared polymer exhibited optically active.

* Corresponding author. Tel.: +886 2270 17147; fax: +886 2270 25238.
E-mail address: smchen78@ms15.hinet.net (S.-M. Chen).

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Nile blue A (NB), one of phenoxazine dyes, is a well-known electroactive molecule. It has been used as a mediator for electron transfer with a two-electron redox conversion to modify solid electrodes and used for electrocatalytic applications [31–33]. The incorporation of phenothiazine [34] and phenoxazine dyes [15,35] into DNA modified electrode have been reported. Recently, Hu et al. [35] studied the interaction between Nile blue and immobilized single- or double-stranded DNA and its application in electrochemical recognition and calculated binding constants. But, they have not been used electrode to electrocatalytic application.

Cytochrome *c* (Cyt *c*) plays a major role in electron transport in biochemical transformations. It is a water-soluble heme protein that exists in the cytosol between the inner and outer membranes of mitochondria. Under physiological conditions, it transfers electrons between cytochrome *c* reductase and cytochrome *c* oxidase, which are both embedded in the mitochondrial membrane. Reversible electron transfer between electrode and redox proteins immobilized in films provides a basis for constructing biosensors, biomedical devices, and enzymatic bioreactors [36]. Many reports have described the electrochemistry of cytochrome *c* in terms of modifier electrode and modifier-protein interactions [37–40]. Many promoters, such as some small organic compounds [41–42], small peptides [43] and conductive polymers [44] have been found to promote the direct electrochemistry of cytochrome *c* on the electrode surface. Various dyes associated with electrode surfaces have been proven to align cytochrome *c* and to facilitate interfacial electron transfer [45–46].

In the present work, we report that the amplified electrochemical sensing of DNA is accomplished by electrodeposited PEDOT on the electrode surfaces and incorporation of Nile blue (NB) as redox active intercalator into DNA. This modified composite electrode is utilized towards catalytic reduction of hydrogen peroxide. To the best of our knowledge, there is no literature available on elsewhere.

2. Experimental

2.1. Reagents and solutions

EDOT, DNA, and NB were purchased from Aldrich. All reagents were of analytical grade and used without any further purification. Solutions were prepared with doubly-distilled water. High purity nitrogen was used for deaeration. The buffer and sample solutions were purged with highly purified nitrogen for at least 10 min prior to the experiments. Nitrogen atmosphere was maintained over the solutions during the experiments to prevent the reentry of atmospheric oxygen.

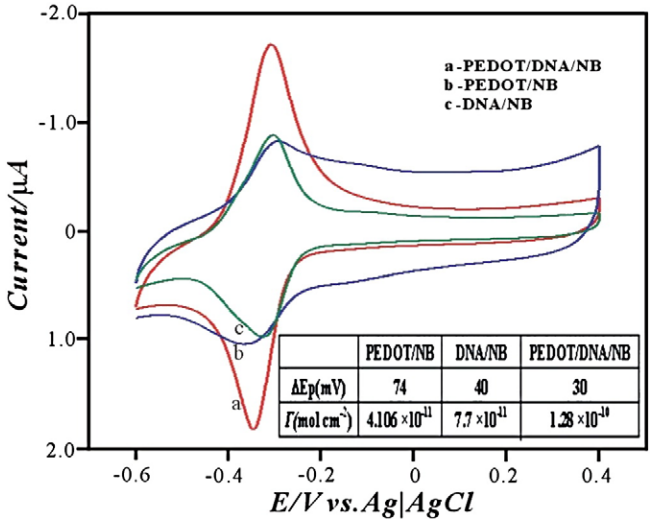

Fig. 1. CVs of (a) PEDOT/DNA/NB composite (b) PEDOT//NB (c)DNA/NB electrode. Electrolyte; 0.2 M PBS solution, pH:7: scan rate: 50 mV/s.

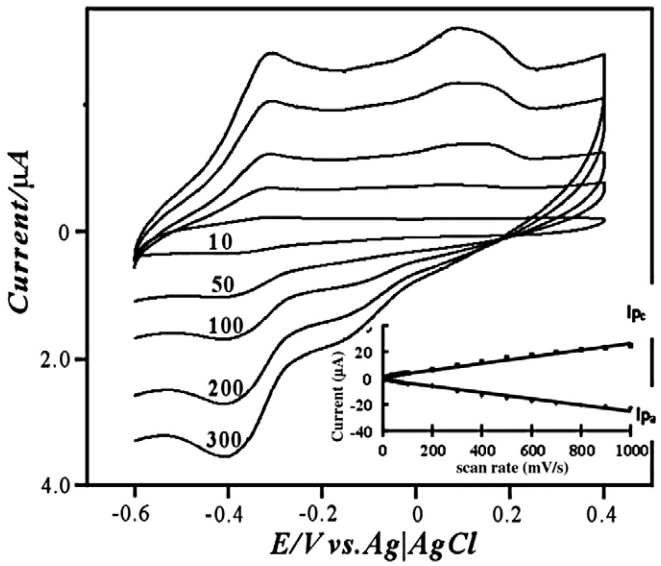

Fig. 2. CVs of PEDOT/DNA/NB composite electrode at different scan rates: scan rate: (a) 0.01 (b) 0.05 (c) 0.1 (d) 0.2 (e) 0.3 (V/s): Inset: scan rate vs Ipa/Ipc. Electrolyte; 0.2 M PBS solution, pH 7.

2.2. Apparatus

Electrochemical experiments were performed with CH Instruments (Model CHI-400) using CHI-750 potentiostat. Glassy carbon electrode (geometric area 0.07 cm^{-2}) obtained from BAS served as the working electrode. Pt wire was used as counter electrode and Ag/AgCl with the saturated KCl solution used as reference electrode. All the potentials given in this paper were referred Ag/AgCl (saturated KCl solution).

2.3. Preparation of PEDOT/DNA/NB composite electrode

Prior to modification, glassy carbon electrode (GCE) was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and used. After being cleaned, the polished GC surface was subjected to electrochemical deposition of poly(3,4-ethylenedioxythiophene) (PEDOT) film (three cycles within the potential range −0.5 to 1.1 V) from the solution containing 0.01 M EDOT, and 0.1 M $LiClO_4$ potentiodynamically. The 20 μl of 2 mg DNA solution was spread over the PEDOT modified electrode and dried to get the film

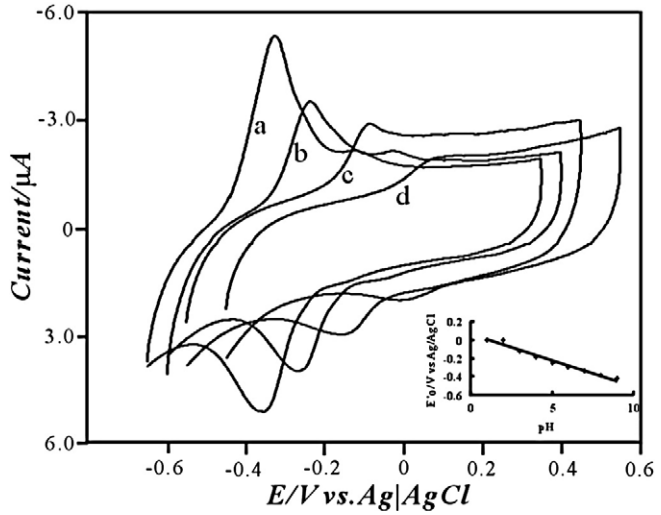

Fig. 3. CVs of different pH (a) 7 (b) 5 (c) 3 (d) 1. Scan rate: 50 mV/s. Inset: pH vs E°′.