Biosensors and Bioelectronics 69 (2015) 100–105

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A label-free electrochemical DNA biosensor based on covalent immobilization of salmonella DNA sequences on the nanoporous glassy carbon electrode

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ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form
12 February 2015
Accepted 13 February 2015
Available online 16 February 2015

Keywords:
Nanoporous glassy carbon electrode
Salmonella
Covalent immobilization
DNA biosensor
Label-free detection

ABSTRACT

Herein, an easy and cost-effective approach to the immobilization of probe was performed. The amino modified salmonella ssDNA probe sequence was covalently linked with carboxylic group on the surface of nanoporous glassy carbon electrode to prepare the DNA biosensor. The differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) techniques were used for the determination of salmonella DNA in the concentration ranges of 10–400 pM and 1–400 pM with limits of detection of 2.1 pM and 0.15 pM, respectively.

1. Introduction

Food borne diseases are a major public health and economic problem in the world. The Centers for Disease Control and Prevention (CDC) reported that only in the United States of America over 48 million people are sickened as a result of consuming contaminated foods or beverages (Scharff, 2011). According the European Centre for Disease prevention and Control (ECDC), salmonellosis is the second most common cause of food poisoning after campylobacter. The Economic Research Service (ERS) estimates that the annual economic cost of salmonellosis is 4.430 billion dollars (Scharff, 2011). Salmonella bacteria has been found in un-pasteurized milk, eggs, raw egg products, meat and poultry (Hoelzer et al., 2011). Therefore, it is necessary to develop a sensitive, selective and cost-effective method for determination of salmonella bacteria.

Recently, DNA detection methods have attracted considerable interest due to their early diagnosis of diseases. Up to now, numerous DNA detection methods have been reported in literature for the determination of salmonella, such as polymerase chain reaction (PCR) (Ma et al., 2014; Margot et al., 2013), rolling circle amplification (RCA) (Sato et al., 2013, 2010) and nucleic-acid sequence-based amplification (NASBA) (Mollasalehi and Yazdanparast, 2012, 2013). However, these methods are generally time-consuming, complex and expensive operations. To overcome these shortcomings, the electrochemical DNA biosensors are especially promising because of their simplicity, high sensitivity and selectivity (Erdem et al., 2006; Ligaj et al., 2014; Singh et al., 2013). It is well known that the electrochemical behaviors of DNA biosensors are related to the physico-chemical properties of the electrode surfaces. Several materials for the fabrication of electrochemical DNA biosensor have been reported in the literature (Hu et al., 2011; Peng et al., 2009; Rai et al., 2012; Yang et al., 2007; Zhang et al., 2013). Among them, the carbon based nanomaterials not only provide a large microscopic surface area for immobilization of bio-molecules, but also introduce a desirable biocompatible microenvironment (Gupta et al., 2013; Hu et al., 2011; Huang et al., 2014; Kim et al., 2012; Wang et al., 2011; Wei et al., 2010; Yola et al., 2014; Zhou et al., 2009). The nanoporous glassy carbon is one of these carbon based nanocomposites. The application of nanoporous glassy carbon electrode for the fabrication of electrochemical sensor (Geremedhin et al., 2013; Xu et al., 2010; Zhao et al., 2009) and biosensor (Haghighi and Tabrizi, 2011; Zhang et al., 1996) has been reported previously. To the best of our knowledge, the use of a nanoporous glassy carbon electrode for immobilization of DNA and its application as a DNA biosensor has not been yet reported. The DNA biosensor proposed in this work exhibited excellent electrochemical characteristics and high analytical performance in terms of sensitivity, stability, selectivity, linear range and limit of detection.

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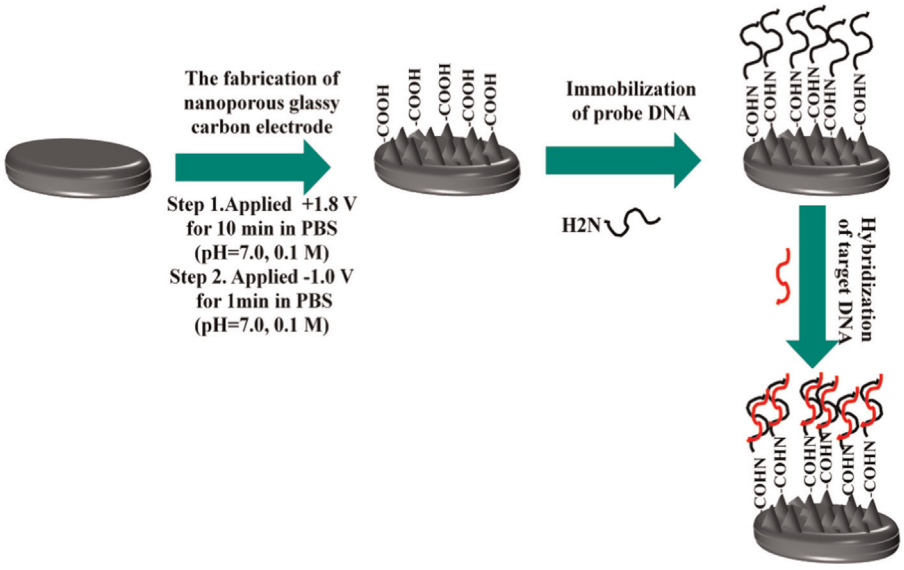
http://dx.doi.org/10.1016/j.bios.2015.02.024
0956-5663/

2. Experimental

2.1. Reagents and chemicals

All chemicals were of analytical reagent grade and used without further purification. Potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), potassium chloride (KCl), hydrochloric acid (HCl), potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) and potassium hexacyanoferrate(II) ($K_4[Fe(CN)_6]$) were obtained from Merck (Darmstadt, Germany). N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride $C_8H_{17}N_3$.HCl (EDC.HCl) and N-hydroxysuccinimide (NHS, 98%) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Double distilled water was used in all experiments. DNA oligonucleotides with the following sequences were obtained from Fazabiotech Co. (Tehran, Iran):

Probe single strand DNA (ssDNA):NH_2-5′-GGA GCT GCT GGC ATT ATT GAA-3′
Target DNA: 5′-TTC AAT AAT GCC AGC AGC TCC-3′



Scheme 1. The schematic illustration for fabrication of DNA biosensor.

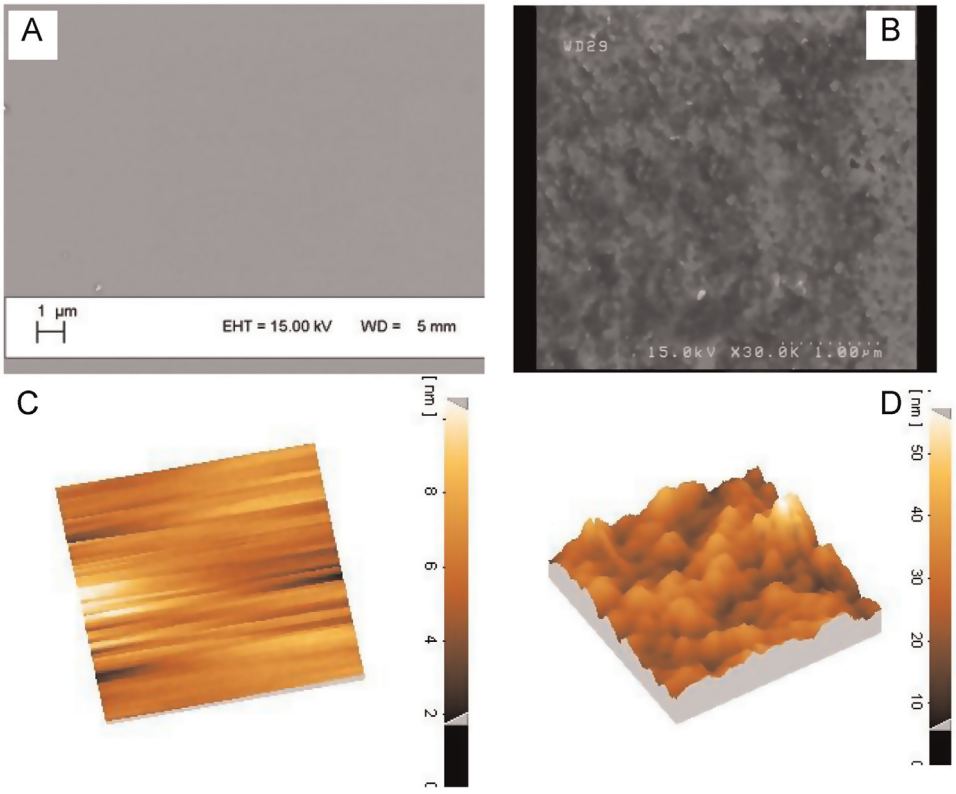


Fig. 1. SEM and AFM images of polished GCE (A,C) and the prepared GCE-red (B,D).