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Nanostructured rough gold electrodes as platforms to enhance the sensitivity of electrochemical genosensors

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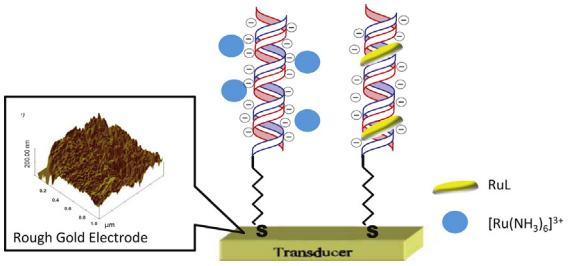
HIGHLIGHTS

- Nanostructured rough gold electrodes as general DNA biosensors platforms.
- Electrode nanopatterning was accomplished by repetitive square-wave perturbing potential.
- Hybridization was electrochemically monitored by using electrostatic or intercalative indicators.
- PCR amplified real samples can be detected without complicated sample treatment procedures.
- The genosensor exhibits a wide linear range, high sensitivity and remarkable long-term storage stability.

GRAPHICAL ABSTRACT



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ABSTRACT

An electrochemical DNA genosensor constructed by using rough gold as electrode support is reported in this work. The electrode surface nanopatterning was accomplished by repetitive square-wave perturbing potential (RSWPP). A synthetic 25-mer DNA capture probe, modified at the 5′ end with a hexaalkylthiol, able to hybridize with a specific sequence of *lacZ* gene from the *Enterobacteriaceae* bacterial family was assembled to the rough gold surface. A 25 bases synthetic sequence fully complementary to the thiolated DNA capture probe and a 326 bases fragment of *lacZ* containing a fully matched sequence with the capture probe, which was amplified by a specific asymmetric polymerase chain reaction (aPCR), were employed as target sequences. The hybridization event was electrochemically monitored by using two different indicators, hexaammineruthenium (III) chloride showing an electrostatic DNA binding mode, and pentaammineruthenium-[3-(2-phenanthren-9-yl-vinyl)-pyridine] (in brief RuL) which binds to double stranded DNA (dsDNA) following an intercalative mechanism. After optimization of the different variables involved in the hybridization and detection reactions, detection limits of 5.30 pg μL^{-1} and 10 pg μL^{-1} were obtained for the 25-mer synthetic target DNA and the aPCR amplicon, respectively. A RSD value of 6% was obtained for measurements carried out with 3 different genosensors prepared in the same manner.

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1. Introduction

The use of nanostructured electrode platforms for improving the sensitivity, selectivity and analytical performance of electrochemical sensors and biosensors constitutes a recent trend in bioelectroanalytical chemistry [1,2]. This enhanced electroanalytical behavior has been attributed to the fascinating properties of nanostructured electrodes such as superior conductivity, large surface area, high stability and biocompatibility. In the particular case of nucleic acid sensors, the improvements in sensitivity can be attributed to increased capture sites as a consequence of the larger surface area provided by nanostructured electrodes. Furthermore, surface nanostructuration may play an important role in the orientation and assembly density control of probe DNA for optimized hybridization recognition ability [3–6]. In this context, it has been reported recently the dramatic enhancement of target accessibility during hybridization to nucleic acid probes attached on a nanostructured electrode surface leading to faster and more efficient binding compared to tethering of the same probe on a smoother surface. The authors also pointed out on the benefits of a high degree of nanostructuring for overcoming the negative influence of a dense probe packing on the hybridization efficiency [2,4,7].

Nanostructuration of electrode surfaces can be accomplished following different strategies such as modification of the surface with the corresponding nanomaterial or surface nanopatterning. This latter approach can be applied to gold electrodes by electroreduction of thick accumulated oxide layers through application of a repetitive square-wave perturbing potential (RSWPP) in acid solution [8]. The resulting nanopatterning produces rough electrodes with a large active surface area, which can be profited for the preparation of bioelectrodes with improved analytical performance. In this context, we reported recently the preparation of a lactate enzyme biosensor with a nanostructured rough gold surface obtained by an easy, time-saving and controllable protocol [9]. The good analytical performance exhibited by the lactate biosensor suggested that rough gold electrode surfaces can be considered as a promising electrochemical transducer for the development of other bioanalytical platforms. Taking advantage of these important features, DNA biosensors based on nanostructured gold electrodes have been developed recently for the detection of synthetic short DNA sequences [10], characteristic genes fragments such as PML/RARα fusion gene [11] or osteosarcoma-related gene [12] and bacterial DNA [13].

Therefore, we decided to use this approach to develop a novel genosensor for the detection of a characteristic gene (*lacZ* gene) of the *Enterobacteriaceae* family. The methodology involved the use of rough gold electrodes prepared by RSWPP for immobilization of a suitable specific thiolated probe. The development of rapid, reliable and sensitive analytical methods for the detection of microorganisms has attracted great interest in last years due to their relevance in fields such as water and food contamination. An appropriate indicator for routine analysis of this kind of samples is the bacterium *Escherichia coli* (*E. coli*) [14,15]. Although most *E. coli* strains are harmless, their presence in water may be indicative of deterioration of the microbial quality of water, fecal contamination, and the possible existence of other pathogenic microorganisms [16]. Conventional methods for the detection of *E. coli* are laborious and time consuming. Therefore, a number of more rapid methods have been proposed [17] including those based on genetic characterization lead to unequivocal species identification [18]. In particular, genetic methods based on hybridization are very effective on sequence-specific DNA detection. In this context, DNA biosensors based on the integration of a sequence-specific probe and an electrochemical signal transducer are nowadays considered as very attractive analytical tools due to their simplicity, low cost and possibility for real time accurate detection with low detection limits [19,20].

Table 1
DNA sequences used in this work.

Oligonucleotides sequences (5′→3′)	
lacZ-SH	$SH(CH_2)_6$-CAGGATATGTGGCGGATGAGCGGCA
ClacZ	TGCCGCTCATCCGCCACATATCCTG
NC*lacZ*	CATTATGTCGTATAAGTCGCGAACT
lacZ forward primer	ATGAAAGCTGGCTACAGGAAGGCC
lacZ reverse primer	GGTTTATGCAGCAACGAGACGTCA

This manuscript reports for the first time the application of a nanostructured gold electrode for the direct hybridization detection of a predominantly ssDNA *lacZ* gene 326-bp fragment amplified by asymmetric PCR (aPCR) which produced predominantly single-stranded (ss)–amplicons, paving the way for the future development of a procedure for the sensitive and specific detection of the relevant *Enterobacteriaceae* bacterial family. The direct hybridization detection of the resulting amplicons was approached using a pentaamin ruthenium [3-(2-phenanthren-9-yl-vinyl)-pyridine] complex (RuL) [21–24] or a commercially available hexaammineruthenium (III) complex as redox indicators and differential pulse voltammetry (DPV) as the transduction technique. RuL interacted with DNA in an intercalative mode [22] while the interaction of the hexaammineruthenium (III) complex with DNA is electrostatic, which led us to perform a comparative study of the analytical behavior of both approaches.

2. Experimental

2.1. Chemicals

Potassium nitrate and sodium chloride were obtained from Scharlab. Anhydrous dimethylformamide (DMF) was obtained from SDS. $K_4Fe(CN)_6$, $K_3Fe(CN)_6$, 6-mercapto-1-hexanol (MCH), hexaammineruthenium (III) chloride and all other chemicals used in this work were reagent grade quality, purchased from Sigma–Aldrich, and used as received without further purification.

Synthetic oligonucleotides (see Table 1) were purchased from Sigma Genosys. Table 1 also includes the primers employed for the amplification of a 326-base region of the *E. coli lacZ* gene using aPCR. The selection of these sequences was based on literature data [25–27].

DNA stock solutions were prepared in Tris-EDTA buffer (TE buffer: 10 mM Tris–HCl, pH 8.0 containing 1 mM EDTA). The final concentration of the probe and the single-stranded (ss)-amplicon solutions was determined by UV–vis molecular absorption spectrometry using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer (NanoDrop Technologies).

Pentaamin ruthenium [3-(2-phenanthren-9-yl-vinyl)-pyridine] complex (RuL) was prepared *in situ*, as it was described in a previous paper [28] and using stock solutions (typically 2.0 mM) of the precursors $[Ru(NH_3)_5(H_2O)]^{2+}$and 3-(2-phenanthren-9-yl-vinyl)-pyridine (denoted as L) prepared in water and dimethylformamide, respectively. 2 mM stock solutions of hexaammineruthenium (III) chloride were prepared also in deionized water.

All solutions were prepared just prior to use. Water was purified with a Millipore Milli-Q purification system (18.2 MΩ cm).

2.2. Apparatus and electrodes

Electrochemical measurements were carried out at room temperature using an Autolab PGSTAT 30 potentiostat from Eco-Chemie and the software package GPES 4.9 (General Purpose Electrochemical System). An electrochemical cell with a three-electrode set-up including the rough gold working electrode (99.99% purity, 1 mm-ϕ, geometric area = 0.06 cm^2) a gold counter