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Label-free electrochemical DNA sensor using “click”-functionalized PEDOT electrodes ☆

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ABSTRACT

Here we describe a label-free electrochemical DNA sensor based on poly(3,4-ethylenedioxythiophene)-modified (PEDOT-modified) electrodes. An acetylene-terminated DNA probe, complementary to a specific “Hepatitis C” virus sequence, was immobilized onto azido-derivatized conducting PEDOT electrodes using “click” chemistry. DNA hybridization was then detected by differential pulse voltammetry, evaluating the changes in the electrochemical properties of the polymer produced by the recognition event. A limit of detection of 0.13 nM was achieved using this highly selective PEDOT-based genosensor, without the need for labeling techniques or microelectrode fabrication processes. These results are promising for the development of label-free and reagentless DNA hybridization sensors based on conducting polymeric substrates. Biosensors can be easily prepared using any DNA sequence containing an alkyne moiety. The data presented here reveal the potential of this DNA sensor for diagnostic applications in the screening of diseases, such as “Hepatitis C”, and genetic mutations.

1. Introduction

Generally, DNA sensors are based on DNA hybridization. In this approach, a single-stranded DNA (ssDNA) probe is immobilized on a surface and exposed to a sample containing the specific complementary target sequence, which is captured by forming a double-stranded DNA (dsDNA) molecule. This recognition event (hybridization) is then transduced into a readable signal. A variety of transduction techniques can be used to monitor this process, including optical (Ma et al., 2013; Yan et al., 2014), mass-sensitive (García-Martinez et al., 2011), and electrochemical methods (Lazerges and Bedioui, 2013).

Electrochemical DNA sensors are reliable, fast, simple, and cost-effective devices that convert the hybridization occurring on an electrode surface into an electrical signal by means of direct or indirect methods. DNA sensors based on indirect methods require the use of labels or electroactive indicators, such as ferrocenyl derivatives (Nakayama, 2002), redox-active enzymes (Patolsky et al., 2001), nanoparticles (Ting et al., 2009), and redox intercalators (Ferapontova and Gothelf, 2009; Millan and Mikkelsen, 1993). Strategies involving labels are time- and labor-consuming and they do not allow real-time detection of target-probe coupling. In contrast, direct detection methods are based on the intrinsic electroactivity of DNA, mostly derived from the oxidation of guanine or adenine bases (Karadeniz et al., 2003; Kerman et al., 2003), and they allow reagentless and simpler detection. However, this direct detection still has some drawbacks, such as its dependence on the number of guanine residues and the need for high oxidation potentials, which may generate side oxidation reactions. Electrochemical impedance spectroscopy (EIS) has also been used as direct technique for DNA detection (Park and Park, 2009). Even though this technique is highly sensitive, this advantage sometimes limits its application as a result of being liable to respond to

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☆ In memory of Dr. Günther Götz.

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interferences too. Nevertheless, it has been successfully replaced by other less sophisticated electrochemical techniques, such as differential pulse voltammetry or chronopotentiometry (Arora et al., 2007). Alternatively, electrochemical DNA sensors based on conducting polymers (CPs) have been used to directly detect DNA hybridization events in a label-free format. The electronic structure of CPs is highly sensitive to environmental changes occurring at the polymer surface, like those generated by a hybridization event (Garnier, 1989; Peng et al., 2009; Prabhakar et al., 2008).

Functionalized CPs are synthesized using pre- and post-functionalization strategies. The former consists of linking the desired functional biomolecule to the corresponding monomer, followed by its polymerization. However, instability or possible damage to oligonucleotides under electropolymerization conditions makes the post-functionalization strategy more suitable for the immobilization of DNA sequences onto CP surfaces. In the latter approach, the substrate is first electropolymerized from a solution containing precursor monomers modified with reactive groups and then subjected to a coupling reaction with a modified DNA probe at the polymer surface.

DNA probes are commonly functionalized with amino or carboxyl groups and then covalently attached to the polymer surface through peptide bonds using carbodiimide coupling chemistry (Peng et al., 2007, 2005). However, although widely used, these reactions are not fully chemoselective in aqueous solvents, and hydrolysis occurs along with the desired coupling reaction, thereby lowering the efficiency of the immobilization. Thus, a more chemoselective coupling reaction would be more advantageous. In this respect, the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides with terminal alkynes, frequently referred to as the "click" reaction, shows high reliability, specificity, and biocompatibility and has been successfully used in post-functionalization reactions.

A few years ago Bäuerle at al. reported the first example of post-functionalization of a novel conducting poly (azidomethyl-EDOT) (azido-PEDOT) by "click" chemistry with various types of terminal alkynes (Bu et al., 2008). Since then, several examples of derivatization of azido-PEDOT with alkyne-containing fluorophores (Daugaard et al., 2008), ferrocene, glycosides, or fullerenes (Bu et al., 2011) have been described. However, to the best of our knowledge, no study has yet applied "click" chemistry to develop label-free DNA hybridization sensors based on conducting PEDOTs.

Here we report on the first voltammetric genosensor based on azidomethyl-derivatized PEDOT electrodes for the label-free detection of a sequence correlating with the "Hepatitis C" virus (HCV). PEDOT was selected as CP due to the simplicity of EDOT monomer functionalization and to its high electrochemical stability (Bu et al., 2008; Kros et al., 2005). An acetylene-terminated oligonucleotide probe, complementary to a HCV target sequence, was immobilized onto an azido-PEDOT polymer by covalent binding using "click" chemistry. DNA hybridization was detected by differential pulse voltammetry (DPV), directly measuring changes in the electrochemical properties of the polymer triggered by the recognition event. We characterized the selectivity of the sensor and the limit of detection (LOD) was determined to fall in the nanomolar range.

2. Materials and methods

2.1. Materials

Azidomethyl-substituted 3,4-ethylenedioxythiophene (azido-EDOT) was synthesized following the protocol developed by Bu et al. (2008). 99.8% anhydrous dichloromethane (DCM) and tetrabutylammonium hexafluorophosphate (TBAPF6) were supplied by Sigma-Aldrich Co. (USA). Gold working electrodes (disk diameter 1.6 mm) were purchased from BASi (Indiana, USA). Dimetyl sulfoxide (DMSO), tris [(1-benzyl-1H-1,2,3-triazol-4-yl)methyl)]amine (TBTA), N,N-diisopropylethylamine (DIPEA), and copper iodide (CuI) were supplied by Sigma-Aldrich Co. (USA). Phosphate-buffered saline (PBS) and tris(hydroxymethyl)aminomethane were purchased from Sigma Aldrich Co (USA) and Panreac Química S.L.U. (Spain), respectively. A 21-mer oligonucleotide related to the "Hepatitis C" virus (HCV-probe) bearing an acetylene group was synthesized by solid-phase methodology, using the phosphoramidite derivative of hex-5-yn-1-ol, according to the protocol reported by the authors of the work Alvira and Eritja (2007). This DNA sequence is complementary to a specific "Hepatitis C" DNA sequence (base location: 8245-8265). The complementary and non-complementary sequences, used to study the selectivity of the sensor through their hybridization with the probe, were provided by Sigma-Aldrich Co. (USA). All DNA sequences listed in Table 1 were supplied as lyophilized powder. All solutions were prepared using Milli-Q water.

Table 1
List of oligonucleotides. *X* represents the acetylene group and the five next *T* bases were introduced as spacers.

DNA sequences	
Probe (HCV-probe)	5'-*XTT TTT* TGG GGA TCC CGT ATG ATA CCC-3'
Complementary target (HCV-target)	5'-GGG TAT CAT ACG GGA TCC CCA-3'
Non-complementary sequence 1 (Nc1-target)	5'-CTC GAT GAC TCA ATG ACT CG-3'
Non-complementary sequence 2 (Nc2-target)	5'-CCC GCA CTT CAC CAC TCC TCA CCA CTT CAC GCC C-3'

2.2. Instrumentation

Electrochemical measurements were performed on an AUTOLAB PGSTAT 30 electrochemical analysis system (Eco Chemie, The Netherlands). Cyclic voltammetry (CV) and DPV experiments were conducted in a three-electrode electrochemical cell, which consisted of a gold working electrode, a platinum wire as counter electrode, and a Ag/AgCl–NaCl (3M) reference electrode. For electrochemical polymerization in DCM, a Ag/AgCl pseudoreference electrode was used and referenced after each use against ferrocene–ferricenium (Fc/Fc$^+$).

Polymer oxidation signals (between 0.5 V and 1 V vs. Ag/AgCl) were measured by DPV. The oxidation current intensity after background current correction was used as analytical signal. Raw DPV data were treated with the GPES 4.7 software package, using the Savitzky and Golay filter, followed by the moving average baseline correction (peak width of 0.01) provided by the software.

Synthesis of HCV-probe bearing an acetylene group was performed on an Applied Biosystems model 3400 DNA synthesizer.

2.3. Preparation of azido-PEDOT electrodes

Gold electrodes were carefully polished with diamond paste and alumina powder of different grain sizes (from 1 μm to 0.05 μm) prior to use. The electrodes were then washed in ultrasonic baths of acetone and ethanol. Electropolymerization of azido-EDOT was performed on the electrodes by CV, using 1.5 mM azido-EDOT monomer and 100 mM TBAPF$_6$ in DCM under argon atmosphere. CV between -1.5 V and $+1.2$ V (vs. Fc/Fc$^+$) at 100 mV/s was applied on the electrodes, resulting in the azido-PEDOT coverage.