New Ferrocene-Functionalized Cationic Polythiophene for Label-Free Electrochemical DNA Detection

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ABSTRACT

A new electrochemical DNA biosensor using gold-bound peptide nucleic acid (PNA) probes and a simple electrostatic method is reported. Indeed a water-soluble, electroactive, cationic polythiophene was synthesized allowing the specific and sensitive detection of unlabeled target nucleic acid on solid surfaces at room temperature. This new analytical method for DNA hybridization detection is specific enough for discrimining one mismatch DNA from the perfect complementary strand and at the moment a sensitivity of 5 femtomoles can be reached.

Keywords: DNA biosensor, PNA, conjugated polymer, cationic polythiophenes.

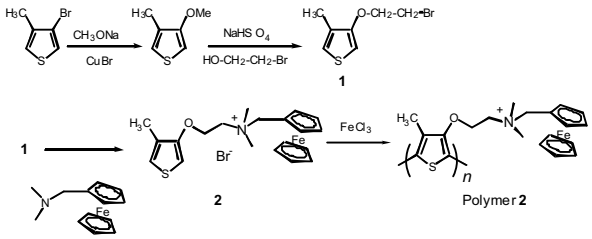
SCOPE

Many efforts have been made worldwide to develop and improve electrochemical DNA methods because of the possibility of making small portable devices and lowering the technology cost. In spite of many advances in this field, e.g. by using gold nanoparticles, enzymes, electrogenerated chemiluminescence, catalytic oxidations, intercalators or conducting polymers, there is still a challenge to find new approaches in order to improve sensibility, selectivity, and simplicity to respond to demands in modern medical diagnostics and biomedical research applications [1].

1 MAKING A DNA BIOSENSOR

1.1 Building elements

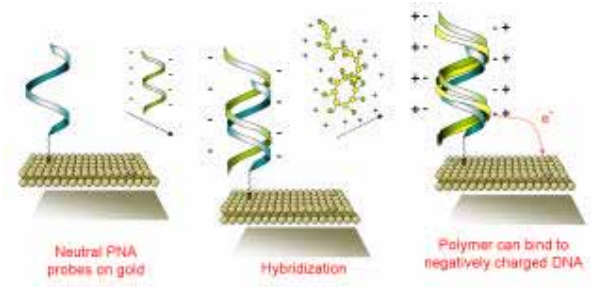
Oligonucleotide-functionalized conjugated polymers have already enabled transduction of hybridization events into an electrical signal without labeling of the DNA target [2-4]. However, this strategy using water-insoluble electroactive polymers modified electrodes leads to a strong electrical background and in those systems hybridization often leads to a decrease of the electrical signal. To solve these problems and to develop a multi-parametric biosensor, we propose a new solid-state electrostatic approach based on neutral peptide nucleic acid (PNA) capture probes [5,6] and an electroactive, cationic, water-soluble polythiophene transducer [7-9].



Scheme 1: Ferrocene functionalized polycationic polythiophene synthesis

The detection of genetic sequences involved the synthesis of a new cationic polythiophene bearing one ferrocene group on each monomeric unit. Synthesis of this new polymer (scheme 1) is quite straightforward: the coupling reaction between compound **1** and the commercial ferrocene derivative leading, in one step and with a quantitative yield, to the desired electroactive unit.

1.2 Working description



Scheme 2: Illustration of the DNA biosensor

To implement this new solid-state electrochemical approach, we first prepared gold electrodes having a monolayer of PNA capture probes. The cationic, water-soluble, and electroactive polymer **2** (Scheme 1) does not bind to neutral PNA capture probes alone but strongly interacts with the negatively-charged backbone of the complementary oligonucleotide bound to PNA probes, allowing transduction of room-temperature hybridization into an electrical signal (Scheme 2). Then, by combining gold-attached PNA probes and electroactive, cationic

polythiophene transducer, we made a simple and sensitive electrostatic method which enables direct detection and specific identification of the complementary nucleic acid analyte without any redox labeling of the probe or the target [10].

2 RESULTS

To determine the presence of the polymer electrostatically bound to the gold surface and to know if hybridization has occurred, square-wave voltammetry (SWV) was performed. Due to the conjugated backbone of the polythiophene and the stable reversible oxidation of ferrocene to ferricinium, a sharp anodic peak is electrochemically observed when hybridization has taken place.

2.1 Optimization and grafting quality control

Probe density is the key element to make the better sensor as possible. Too much probes onto the electrode hinder a good hybridization, whereas too less could permit some non-specific adsorption.

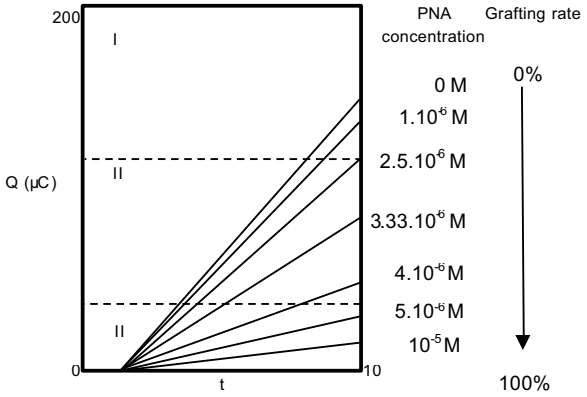


Figure 1: Chronocoulometric responses of different PNA grafting rates on gold electrodes. Electrochemistry was performed under not limited-current diffusion in a highly concentrated solution of H_2O, $Fe(CN)_6^{4-}$ 0,1M. Charge was measured after potential was stepped from Ei=0mV to Ef=550mV. The highlighting (zone II) correspond to the best observed ratio sensitivity/selectivity.

The PNA concentration used as probe on gold electrode was optimized using chronocoulometry and testing of different responses for concentrations ranging from 10^{-6}M to 10^{-4}M and different grafting times ranging from 10 min to 24h. By this way, different zones were determined (Figure 1). The first one is associated with a too low density of PNA probes (I). Another one denotes where hybridization takes place (II) and a last one, where PNA density is too high to allow good hybridization (III).

The best results (reproducibility and signal/noise) were obtained using 1µL of 3-4×10^{-6}M of PNA probes to modify the gold electrode surface. According to theoretical estimations, probe density is evaluated to be between 2.9×10^{-11} to 6.3×10^{-11} molcm^{-2}. This new analytical method is also advantageous for quality control test of the electrode grafting.

2.2 Specificity of the biosensor

This biosensor was used with different analytical DNA sequences in order to test its performances. The specificity of this new analytical method for DNA hybridization detection gives no ambiguity. The sensor is able to clearly distinguish one mismatch DNA from the perfect complementary strand (Figure 2).

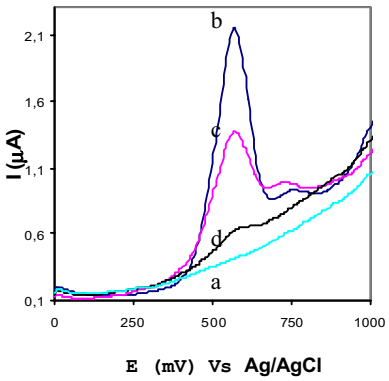


Figure 2: Square-wave voltammograms of PNA modified gold electrode soaked 1 min in a solution of cationic polythiophene: a) Without DNA; b) Perfect match ss-DNA; c) One mismatch ss-DNA; d) Two mismatches ss-DNA.

When the sensor is used with two-mismatch targets, discrimination is more powerful and the observed signal is close to the non-hybridized electrode.

2.3 Sensitivity

The sensitivity of this new electrochemical method for DNA hybridization detection was also tested. When reducing analyte concentration (Figure 3), a decrease of intensity of the Fc/Fc^+ peak is observed.

Peak currents are logarithmically related to target concentrations, with a limit of detection around 5×10^{-15} molecules in a total volume of 10 µL (which is a concentration of 5×10^{-10} M).

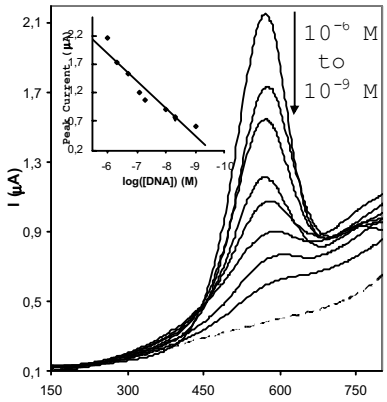


Figure 3: SWV after hybridization at various concentrations of the complementary oligonucleotide, the dotted line represents the background signal in absence of target (insert: variation of intensity vs. logarithm of perfect match target concentration).

This work was done on 2mm^2 surface area gold electrodes. It is expected that further progress in term of sensitivity should be easily obtained by reducing the size of the electrodes and hybridization reaction volumes.

3 PERSPECTIVES

The strategy employing polythiophenes to reveal a negatively-charged surface is adequate to detect and analyze a genomic sequence. To improve the potential biosensor detection, ultramicroelectrodes and interdigitated electrodes will be used to diminish the analytical volumes. As well, ferrocene catalytic activities toward organic products are tested to increase the sensitivity.

In addition, new modified electrodes using cationic polythiophenes and aptamers have been fabricated in order to analyze proteins, another great challenge for medical diagnostics.

4 CONCLUSION

A new electroactive, water-soluble, cationic polythiophene has allowed the specific and sensitive detection of unlabeled DNA targets based on an electrochemical approach with neutral PNA probes. This simple methodology opens interesting possibilities for the future development of integrated, portable, and multiparametric electrochemical device for the diagnosis of infections, identification of genetic mutations, and forensic inquiries. Such objectives will be the subject of future investigations.

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