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Short communication

Detection of DNA hybridization by ABEI electrochemiluminescence in DNA-chip compatible assembly

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Abstract

The electrochemiluminescence (ECL) of a luminol derivate (ABEI) generated both by a carbon electrode and a polypyrrole-coated carbon electrode was examined. It was found that the polypyrrole film (ppy) did not inhibit the ECL. After that, ABEI anchored on a single stranded DNA target (ODNt) has been used for the ECL detection of the hybridization between a complementary single stranded DNA probe (ODNp) covalently linked to a polypyrrole support and the ODNt. The ECL detection has been performed using a DNA sensor having a low surface concentration of ODNp probes, constituted of a polypyrrole copolymer electrosynthesized from a pyrrole-ODNp/pyrrole monomer ratio of 1/20,000.

Keywords: ABEI; ECL; Polypyrrole; DNA; Sensor; Detection

1. Introduction

Electrochemiluminescence (ECL) is a very sensitive method for detecting small amounts of biological substances. This method is widely used in analytical chemistry [1,2] as for the detection of nucleic acid hybridization in DNA biosensors [3]. Very recently, Yang et al. [4] have shown that the recognition between two complementary DNA strands could be detected on a polypyrrole film (ppy) by the ECL of a luminol derivative, *N*-(4-aminobutyl)-*N*-ethyl-isoluminol (ABEI). Their strategy involves a single-stranded DNA used as a target (noted DNAt-ads) and immobilized by adsorption on the surface of a polypyrrole film. A complementary DNA strand bearing the ABEI moiety (noted DNAp-ABEI) was then used as a probe to recognize the target giving the assembly, ppy/DNAt-ads/DNAp-ABEI. The formation of the double-stranded DNA (DNAt-ads/DNAp-ABEI) was successfully detected through the ECL response of the ABEI label anchored to the DNA probe.

In the field of biological analysis, it is essential to detect a series of specific DNA sequences. This could be achieved by using multiparametric assays constituted by various DNA sequence probes linked to the support in a precise location. Thus, the immobilization of DNA targets by adsorption on the polypyrrole film does not appear as a suitable method to the fabrication of a multi-array DNA chip, since the adsorption of DNA targets onto a specific area could not be easily controlled. Also it is not possible to achieve a support bearing a series of DNA targets in a precise location. This approach did not allow the specific DNA sequence detection and thus to elaborate a multiparametric DNA assay in order to analyze an unknown DNA target in solution.

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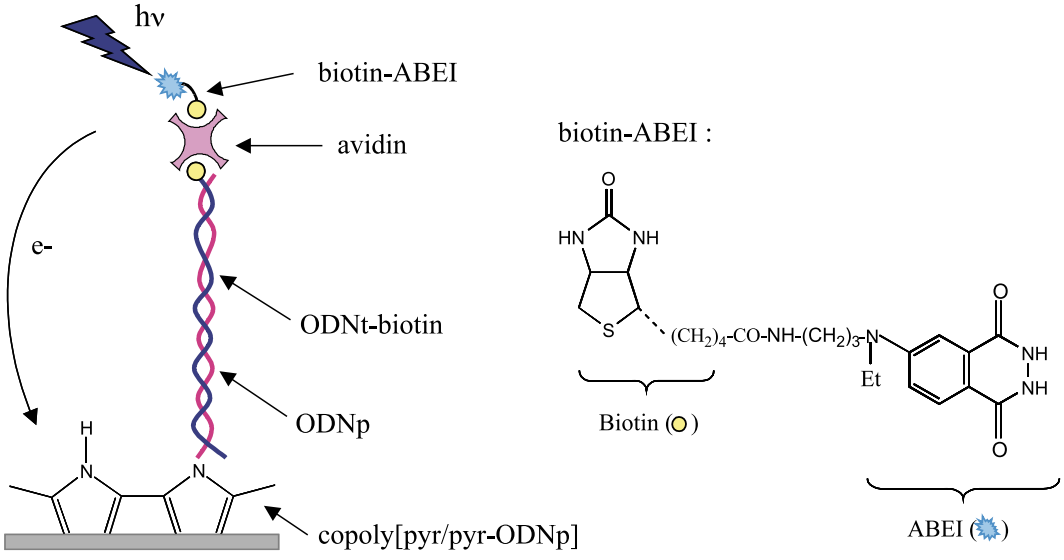
To overcome this, we have developed a method based on the irreversible immobilization of specific DNA sequences (ODN probes noted ODNp) to a polypyrrole film. The grafting of ODN probes is achieved during the electrosynthesis of a copolymer (copoly[pyr/pyr-ODNp]) obtained by the electro-copolymerization of pyrrole units (pyr) and pyrrole monomer bearing ODNp (pyr-ODNp) [5]. This process gives in a single step, a copolymer deposited at the electrode having at its surface ODN probes irreversibly grafted on a polypyrrole chain and available for recognition with complementary strands (ODN targets noted ODNt). As previously described [6], DNA chips have been elaborated by this method from a miniaturized multiparametric device consisting of several different ODN microelectrodes (50×50 μm); each microelectrode being covered by an ODN functionalized polypyrrole copolymer obtained by electropolymerization from a mixture of pyr/pyr-ODNp within a monomer ratio of 1/20,000. This strategy has been validated by the genotyping of C virus [7].

The same elaboration process was used in this work for the construction of the sensing layer in order to study the application of ECL detection in DNA sensor. The aim of this work is to evaluate the usefulness of ECL for the detection of the hybridization between the immobilized ODN probe (ODNp) and the biotinylated ODN target (ODNt-biotin) in solution. For that, a copolymer copoly[pyr/pyr-ODNp] was electrosynthesized on a glassy carbon macroelectrode (S=7×10^{-2} cm^2) with a pyr/pyr-ODNp monomer ratio of 1/20,000. For this monomer ratio, the copolymer is composed of 1 pyr-ODNp for 60,000 pyrrole units [5]. The accessibility level of the ODNp bound to the pyrrole for a complementary ODNt has been accessed to 20% [5]. Then the surface concentration of ODNp probes on the copolymer copoly[pyr/pyr-ODNp] is very low. The ECL label used throughout this work was a biotinylated ABEI (noted biotin-ABEI) grafted to the ODNt-biotin strand through a biotin–avidin link. Also this anchoring method requires first the immobilization of avidin on the ODNt-biotin. It must be noted that this anchoring method of the ECL label based on the biotin–avidin interaction is similar to the one used classically in fluorescence detection using the streptavidin-R-phycoerthrin as marker [6]. Furthermore, since the avidin has four biotin–avidin interaction sites, three sites remains potentially free after its immobilization to the hybridized ODNt-biotin [8]. Then three biotin-ABEI labels were anchored through the avidin to one ODNt-biotin target. By this way, the ECL signal to detect the hybridization between ODNp and ODNt-biotin is amplified. The other focus of this work is the electrical accessibility of the ECL label through the copoly[pyr/pyr-ODNp]/ODNt-biotin/avidin/biotin-ABEI assembly (Scheme 1) and the efficiency of the ECL reaction under biological conditions. In addition, we studied the ECL response of ABEI in solution on a polypyrrole film to determine the effects of polypyrrole on this ECL response. Indeed, it is important to assess the compatibility of the ECL electrical triggering with the polypyrrole chemical and electrochemical stability.

2. Experimental section

2.1. Reagents and apparatus

Avidin, veronal (diethyl barbitural), hydrogen peroxide (30%), $LiClO_4$ and pyrrole were purchased from Sigma-Aldrich. The biotinylated-ABEI was synthesized following a procedure described in the literature [9]. All oligonucleotides were synthesized by Apibio. The pyrrole monomer has been functionalized with an oligonucleotide (ODNp) corresponding to the sequence pyrrole-5′-GCCTTGACGATACAGC-3′.



Scheme 1. Representation of the bio-assembly, copoly[pyr/pyr-ODNp]/ODNt-biotin/avidin/biotin-ABEI, used to test the ECL for the detection of DNA hybridization in a DNA-chips compatible format.

Two biotinylated oligonucleotide sequences were used for testing the specificity of the sensor: the biotinylated complementary oligonucleotide (ODNt-biotin) with the sequence 5′-biotin-GCTGTATCGTCAAGGC-3′ and the biotinylated noncomplementary one (ODNnc) corresponding to the sequence 5′-biotin-ACGCCAGCAGCTCC-3′. Water was purified by a UHQ water purification system (USF-ELGA). The PBS buffer solution contains 0.01 M phosphate buffer+2.7 mM KCl+13.7 mM NaCl (pH=7.4 at room temperature) and was prepared by dissolution of one tablet of PBS in 200 ml of water. The hybridization buffer solution contains 9.5 mM PBS+0.15 mM NaCl+2.6 mM KCl+0.048% Tween+1 part Denhardt's reagent+10 μg ml^{-1} salmon sperm DNA.

The electrochemical apparatus was a Voltalab PGZ 301 from Radiometer Analytical. The electrosynthesis of films were carried out in a conventional three-compartment cell. The working electrode was a glassy carbon electrode ($S=7\times10^{-2}$ cm^2) and the counter-electrode was a platinum wire. A saturated calomel electrode (SCE) acted as a reference electrode.

The ECL intensity measurements (I_{ECL}) were obtained with a luminometer (Biocounter M 2500, Lumac) and signal was recorded with an X-Y recorder (Servotrace, Sefram) or analyzed with a microcomputer to give I_{ECL}–t curves. A spectrophotometer (Spectra Pro 150, Princeton Instruments) in conjugation with the electrochemistry apparatus was used to obtain ECL emission spectra.

2.2. Electrode preparation

The study of the ECL response of ABEI in solution has been realized on a glassy carbon electrode modified by a polypyrrole film. This film was electrosynthesized at +0.8 V vs. SCE from an aqueous solution containing 0.1 M of $LiClO_4$ and 50 mM of pyrrole monomer by passing of the desired electrical charge. The film thickness is assessed from the electrical charge consumed during electrodeposition with 4 nm mC^{-1} cm^2 as conversion factor [10].

The molecular assembly copoly[pyr/pyr-ODNp]/ODNt-biotin/avidin/biotin-ABEI was achieved as follows. First, the copolymer copoly[pyr/pyr-ODNp] was electrosynthesized at +0.8 V vs. SCE by passing of 45 mC cm^{-2} from a solution of lithium perchlorate (0.1 M) containing 20 mM of pyrrole and 1 μM of functionalized pyrrole with oligonucleotide (ODNp). After the synthesis, the modified electrode was thoroughly washed with water and incubated for 20 min at 50 °C in a hybridization buffer containing 20 μM of complementary biotinylated oligonucleotide (ODNt-biotin). Once washed in a PBS solution, this electrode was then dipped for 5 min into a PBS buffer aqueous solution containing 2 g l^{-1} of avidin; the concentration is similar to the one used to elaborate biosensor using the biotin–avidin interaction to anchor biotinylated glucose oxidase enzymes on a avidin layer previously immobilized to a biotinylated polypyrrole film [11]. After washing with PBS buffer, this modified electrode copoly[pyr/pyr-ODNp]/ODNt-biotin/avidin was dipped for 10 min in a PBS solution saturated with biotinylated ABEI to lead to the molecular assembly desired, copoly[pyr/pyr-ODNp]/ODNt-biotin/avidin/biotin-ABEI.

The ECL blank experiment was performed from a glassy carbon electrode modified by the assembly copoly[pyr/pyr-ODNp]/ODNt-biotin/avidin/biotin-ABEI. To do this, the glassy carbon electrode was first coated by the copolymer copoly[pyr/pyr-ODNp] (45 mC cm^{-2}), then incubated successively in a solution containing 20 μM of biotinylated noncomplementary oligonucleotide (ODNnc), 2 g l^{-1} of avidin and finally saturated with biotinylated ABEI.

2.3. ECL measurement

All ECL measurements were realized in 30 mM veronal buffer (pH=10) with 500 μM of H_2O_2. The glassy carbon electrode coated by the desired molecular assembly was polarized at a fixed potential (+1.45 V vs. a platinum electrode) during the entire acquisition time. The ECL intensities obtained are given in arbitrary unit. The experimental set-up used has been this one described in literature [11]. For each experiment, three measurements have been performed. A similar result (with a variation of about 6%) is achieved in all cases proving the good reproducibility of the measurement.

3. Results and discussion

Fig. 1 shows the intensity of the ECL signal measured when applying a potential of +0.6 V vs. SCE during 3 s to a glassy carbon electrode modified with or without a polypyrrole film (15 mC cm^{-2}; thickness: 60 nm) in a

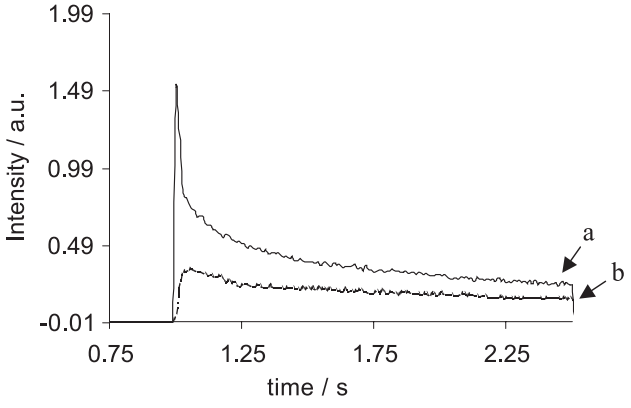


Fig. 1. ECL spectrum observed for a veronal buffer solution containing 0.5 mM of ABEI and 1.3 mM of H_2O_2 (a) on a bare carbon electrode ($S=7\times10^{-2}$ cm^{-2}) and (b) coated with a polypyrrole film (15 mC cm^{-2}).

veronal buffer solution (pH=10) containing ABEI (0.5 mM) and H_2O_2 (1.3 mM). As shown from these curves, both electrodes gave an ECL signal although the deposition of a polypyrrole film leads to a decrease of the ECL intensity. When a potential of +0.6 V was again applied to the polypyrrole modified electrode, the ECL intensity decreased further in contrast to the carbon bare electrode, which showed a stable response. After eight potential pulses, the intensity measured was only 1/100 of the initial one. We attribute this behaviour to over-oxidation of the polymer due to the conditions used to perform the ECL reaction (basic pH of the solution, presence of H_2O_2, associated to the ECL potential value applied). Indeed, after the first ECL measurement, the polypyrrole film shows a much lower electrochemical response as determined by cyclic voltammetry in H_2O+Li-ClO_4 0.1 M. Furthermore, we have observed that the thickness of the underlying polypyrrole matrix does not strongly affect the ECL intensity of ABEI, since the same results have been obtained with a polypyrrole film twice as thick (electrosynthesized with a charge of 31.6 mC cm^{-2}). Finally, it is worth noting that the ECL emission spectrum of ABEI on the carbon electrode is the same as the one recorded with the electrode modified by a polypyrrole film. These simple experiments proved that it is possible (i) to trig the electrochemiluminescence of the ABEI through a polypyrrole film and (ii) to record a measurable ECL signal.

We have then studied the detection of DNA hybridization using the ECL of ABEI from a polypyrrole-based biosensor as previously developed by us [7]. Hybridization studies were carried out with a polymer layer having an average thickness of 100 nm. When the ECL potential was applied to the glassy carbon electrode coated with the molecular assembly copoly[pyr/pyr-ODNp]/ODNt-biotin/avidin/biotin-ABEI, we observed (Fig. 2) the ECL intensity corresponding to 819 arbitrary units (a.u.). In a blank experiment using noncomplementary oligonucleotides (ODNnc), the ECL intensity was only 266 a.u. This was probably due to the nonspecific interaction of biotin-ABEI with the copolymer and/or its anchoring to avidin adsorbed on the film [11].

The results described herein demonstrate that the ECL response of ABEI is not inhibited by the presence of a polypyrrole film coated on the electrode surface. Thus, the ECL phenomenon can be used to detect the hybridization from a DNA sensor elaborated according to parameters used in the strategy of biochip preparation. In addition, it must be emphasize that the process of immobilization of ODN probes followed by the ABEI labelling of the ODN target after hybridization is fully compatible with multiparametric DNA-array implementation. In addition, research in progress in our laboratory shows that the use of a DNA-chip microelectrode array format is compatible with ECL and the signal ratio between ODNp and ODNnc can be greatly improved by a BSA treatment which could drastically reduce the nonspecific adsorption as classically observed in biochemistry.

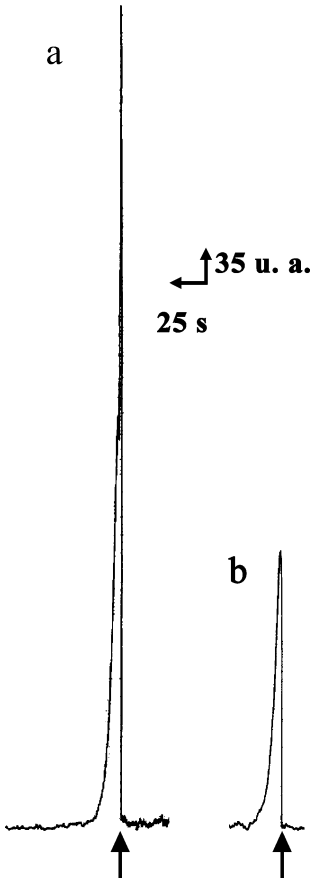


Fig. 2. The I_{ECL}–t curves of biotinylated ABEI on a copolymer film copoly[pyr/pyr-ODNp], after incubation in a solution containing (a) biotinylated complementary ODNt (ODNt-biotin) and (b) biotinylated noncomplementary ODNnc oligonucleotides (see text). The arrows show the moment where the potential was applied to initiate the ECL process.

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