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Electrochemical functionalization of polypyrrole through amine oxidation of poly(amidoamine) dendrimers: Application to DNA biosensor



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ABSTRACT

Electrochemical patterning method has been developed to fabricate composite based on polypyrrole (PPy) film and poly(amidoamine) dendrimers of fourth generation (PAMAM G4). PPy layer was generated using electrochemical polymerization of pyrrole on a gold electrode. PPy film was then modified with PAMAM G4 using amines electro-oxidation method. Covalent bonding of PAMAM G4 and the formation of PPy-PAMAM composite was characterized using Fourier Transform Infrared Spectroscopy (FT-IR) and X-ray Photoelectron Spectroscopy (XPS). Ferrocenyl groups were then attached to such surface as a redox marker. Electrochemical properties of the modified nanomaterial (PPy-PAMAM-Fc) were studied using both amperometric and impedimetric methods to demonstrate the efficiency of electron transfer through the modified PPy layer. The obtained electrical and electrochemical properties were compared to a composite where PPy bearing carboxylic acid functions was chemically modified with PAMAM G4 by covalent attachment through formation of amid bond (PPy-CONH-PAMAM). The above mentioned studies showed that electrochemical patterning does not disturb the electronic properties of PPy. The effect of the number of functional groups introduced by the electrochemical patterning was demonstrated through the association of various compounds (ethylenediamine, PAMAM G2 and PAMAM G6). We demonstrated that such compounds could be applied in the biosensors technology. The modified PPy-PAMAM-Fc was evaluated as a platform for DNA sensing. High performance in the DNA detection by variation of the electrochemical signal of ferrocene was obtained with detection limit of 0.4 fM. Furthermore, such approach of electrochemical patterning by oxidation of amines could be applied for chemical modification of PPy and open a new way in various biosensing application involving functionalized PPy.

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1. Introduction

Polypyrrole (PPy) is one of the conducting polymers extensively applied to the design of bioanalytical sensors [1,2] including systems for DNA detection [3,4]. Immobilization of bioreceptors on PPy film is still an important issue in the use of this polymer for biosensors construction. Literature has reported different strategies, for example DNA probe can be immobilized via entrapment method during electropolymerization process [5]. However, this method can cause unfavourable orientation of molecules in the polymer layer. One of the powerful alternative for stable biomolecules attachment, is the chemical modifications of pyrrole monomer on N-nitrogen or 3-substituted C-carbon position

followed by electrochemical polymerization [6]. This method consists in the modification of pyrrole with functional groups such as carboxylic acids [7], amines [8] or activated esters [9,10] which gives the flexibility for covalent attachment of biomolecules. Nevertheless, construction of these biosensors requires the synthesis of pyrrole modified with functional groups, which is time consuming and laborious.

Association of PPy with structured, three-dimensional materials such as poly(amidoamine) dendrimers (PAMAM) can greatly increase the quantity of attached biomolecules and consequently improve sensitivity of detection [11]. PAMAM are the spherical, hyper-branched, macromolecular polymers, that possess a large number of amine groups on the surface depending on the generation [12]. These molecules have been successfully applied in the fabrication of DNA biosensors using amperometric [13] as well as impedimetric techniques [14,15] showing improved quantity of attached DNA probes to the surface. It was also demonstrated that

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their association with others conductive nanomaterials such as gold nanoparticles or graphene can additionally amplify sensitivity of electrochemical signal and enable a low detection limit [16,17]. Recently, our group has showed the immobilization of PAMAM dendrimers of fourth generation (PAMAM G4) on PPy film modified with activated carboxylic groups using a chemical functionalization of pyrrole monomers. We proved that PAMAM G4 led to high surface coverage of DNA aptamers as bioreceptors improving sensitivity of protein detection [18].

In the current work, we demonstrate that electrochemical oxidation of amines could be applied for the chemical functionalization of conducting materials such as PPy. The advantage of this method is its simplicity and short time, without prior chemical synthesis of the functionalized pyrrole monomer. Such approach of surface functionalization with aliphatic molecules has been previously demonstrated in the case of carbon based materials such as glassy carbon [19–21], carbon nanotubes (CNTs) [22] and polyparaphenylene [23] modified electrode. We recently demonstrated that PAMAM G4 dendrimers could be attached to CNTs-PPy composite via electrochemical method [24] and such material was successfully used for DNA detection. In this work the modification of un-functionalized PPy film by electrochemical deposition of PAMAM G4 is demonstrated. To our knowledge, this is the first time that non-functionalized PPy film was modified with amine terminated macromolecules such as PAMAM G4 through electrochemical oxidation of its amine groups.

The PPy-PAMAM composite was characterized with various techniques and its properties were compared with material obtained following chemical binding of PAMAM G4 to the PPy layer modified with carboxylic acid groups (PPy-CONH-PAMAM). The obtained material was then applied for DNA sensing where sensitivity and selectivity were investigated through ferrocene signal variation attached as a redox marker to the modified surface.

Fabrication of PPy-PAMAM material as well as construction of biosensor is presented on Scheme 1. Steps a and b show electropolymerization of PPy following covalent grafting of PAMAM G4 by its amine moieties. Then ferrocene chemically functionalized with two phthalimidyls was attached to such surface by formation of peptidic bonds with PAMAM G4 (step c). ssDNA probe modified with amine group on 5' position was anchored to unreacted phthalimidyl of ferrocene (step d) and interaction DNA probe/DNA target (step e) was followed by variation in ferrocenyl signal using square wave voltammetry (SWV) technique.

2. Materials and methods

2.1. Reagents

All DNA sequences were provided by Eurogentec company. The single-stranded DNA probe (ssDNA) was an amine terminated 15-bases sequence: $\text{NH}_2\text{-(CH}_2\text{)}_6\text{-5'-GAT-ACT-TCT-ATC-ACC3'}$. The sequence of 15-bases target specific for DNA probe (DNA_C) was 5' $\text{GGT-GAT-AGA-AGT-ATC3'}$. The non-complementary 14-bases oligonucleotide (DNA_NC) sequence was 5' $\text{CAT-TCC-CTC-TTA-GG3'}$.

1,1'-(phthalimide butanoate) ferrocene and pyrrole monomer bearing activated ester were synthesized according to the methods described previously [25,26]. Pyrrole, poly(amidoamine) dendrimers of fourth generation (PAMAM G4) and lithium perchlorate were purchased from Sigma-Aldrich. Prior to polymerization, pyrrole was distilled under argon and PAMAM G4 were purified by 0.22 μm membrane filters (Corning). All the electrochemical analysis were performed in phosphate buffer saline (PBS) pH 7.4 containing 10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 2.7 mM KCl and 137 mM NaCl, filtered through 0.22 μm pore size membrane and stored at 4 °C until use.

2.2. Instrumentation

Electrochemical polymerization was performed using a potentiostat-galvanostat Autolab PGSTAT 12 controlled by Nova software. The three-electrode cell was purchased from Basi and consists of a platinum mesh as a counter electrode, gold disc (surface $2.01 \times 10^{-2} \text{ cm}^2$) as a working electrode and Ag/AgCl as a reference electrode. After polymerization and in between each step of biosensor construction, the modified surface was analyzed by cyclic voltammetry (CV) and square wave voltammetry (SWV) methods in 10 mM PBS buffer pH 7.4. CV was performed in the potential range from -0.6 to 0.4 V with the scan rate of 50 mV s^{-1} . SWVs were performed in the potential range from -0.6 to 0.4 V with conditioning time of 120 s, modulation amplitude of 20 mV and frequency of 50 Hz.

Electrochemical impedance measurements (EIS) were carried out in 10 mM PBS buffer pH 7.4. All impedance measurements were obtained at 0.10 V vs. Ag/AgCl corresponding to the steady state potential with signal amplitude and a sinusoidal potential excitation corresponding to 10 mV is applied in a frequency range from 100 kHz to 0.1 Hz.

Chronoamperometric measurements were also performed in 10 mM PBS containing 5 mM/5 mM $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ at the potential of 0.2 V vs. Ag/AgCl. The current was measured for 90 s.

Fourier Transform Infrared spectra (FT-IR) were measured using a Bruker IFS66 FT-IR spectrometer (Bruker, Germany) equipped with a Mercury-cadmium-telluride (MCT) detector and an attenuated total reflectance (ATR) germanium crystal. The analyses were performed on screen printed electrodes (DropSens).

X-ray Photoelectron Spectroscopy (XPS) measurements were performed with a K Alpha spectrometer from Thermo-Fisher, using a monochromated X-ray Source (Al K α , 1486.6 eV). For all measurements, a spot size of 400 μm was employed. The hemispherical analyser was operated in CAE (Constant Analyser Energy) mode, with a pass energy of 200 eV and a step of 1 eV for the acquisition of surveys spectra, and a pass energy of 50 eV and a step of 0.1 eV for the acquisition of high resolution spectra. The spectra obtained were processed using the "Avantage" software provided by Thermo-Fisher. A Shirley-type background subtraction was used.

2.3. Electrochemical patterning of PPy and PAMAM G4 dendrimers

Polypyrrole film (PPy) was deposited on the gold electrode surface, by cycling the potential from -0.4 to 0.9 V vs. Ag/AgCl, with a scan rate of 100 mV s^{-1} for 2 cycles in H_2O solution containing 0.5 M LiClO_4 . During electro-polymerization, the working and counter electrodes were separated in small volume cell (Basi) containing the solution of 100 mM pyrrole monomer. After reaction, the electrode was rinsed several times with double distilled water.

PAMAM G4 dendrimers were immobilized on the PPy layer by covalent bonding during electro-oxidation of amine groups of PAMAM G4. Electrodeposition was performed in H_2O containing 50 μM PAMAM G4 and 0.5 M LiClO_4 by scanning the potential from 0.0 to 1.1 V vs. Ag/AgCl as a reference electrode during 3 cycles with the scan rate of 50 mV s^{-1} . During the reaction the working and counter electrodes were separated in small volume cell (Basi) similar to electropolymerization method.

2.4. Association of ferrocene and ssDNA probe

Covalent bonding of ferrocene modified with two phthalimidyls was performed by immersing the modified PPy-PAMAM electrode in solution of 1 mM ferrocene prepared in acetonitrile for 1 h at room temperature. The time and concentration of ferrocene were

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