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Characterization of PEDOT film functionalized with a series of automated synthesis ferrocenyl-containing oligonucleotides

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Abstract—In previous works we have described a fully automated synthesis of new ferrocene labelled oligonucleotides (Fc-ODNs) probes with one or more electroactive markers at different position in the chain. These Fc-ODNs have shown good properties to detect ODN target in solution. Here we describe the post-functionalization of a conducting co-polymer based on ethylenedioxythiophene (EDOT) derivatives by a series of Fc-ODNs. The grafting of the Fc-ODNs probes resulted in the appearance of the ferrocene redox couple which directly confirm the effectiveness of the ODN anchoring compared to traditional approach based on IR spectroscopy and X-ray fluorescence of the films. Moreover, the electrochemical response of the modified electrodes analysed in organic media before and after hybridization with ODN target confirm that properties obtain in solution for Fc-ODNs already exist in the film. The changes in the current intensity were found to be dependant on the structure of the grafted ODN that validate our strategy to synthesize an optimal Fc-ODNs.

1. Introduction

The development of efficient methods for real-time detection of specific DNA sequences is currently receiving a tremendous amount of interest for applications in clinical diagnostics[1] environmental protection,[2,3] food quality control,[4] and forensic science.[5] In this context electrochemical sensor devices have emerged as promising tools due to their high sensitivity, easy implementation, low production cost and ability to miniaturization.[6–8] Such systems are based on the covalent immobilization of probe oligonucleotides (ODNs) onto a surface, which allows the direct detection of the hybridization reaction with the target ODN.[9] In that connection, conjugated polymers as polythiophene or polypyrrole did appear as valuable conducting substrates for the grafting of the ODN probes. Indeed the change in either the intrinsic electroactivity of the conducting backbone or the electrochemical properties of a pending redox label can be used as a transduction mechanism to monitor the hybridization reaction with the target ODN.[9–18] However, IR spectroscopy and X-ray fluorescence of the films are generally necessary to confirm the grafting of ODNs[19] and no quantitative determination of the amount of ODN immobilized on the polymer film could be achieved. Moreover, the common functional feature pertaining to these systems is the intensity decrease of the electrochemical signal in response to hybridization, which is detrimental to the sensitivity performance of the sensor.

In this context, we have developed a new strategy based on the post-functionalization of a conducting polymers by ODN probes and ferrocene units at the same place and in a one-pot reaction. Indeed the ferrocene moiety displays a reversible and narrow redox behavior that is sensitive to electronic and steric factors. By this way the appearance of the ferrocene redox couple in cyclic voltammetry (CV) directly confirm the effectiveness of the ODN anchoring and the exact integration of the amount of charge exchanged by ferrocenyl groups allows to the electrode coverage.

In previous works we have prepared and demonstrated the good capacity to detect ODN target of a series of ODNs bearing different number of ferrocene unit directly incorporated into the base sequences during the automated solid phase (Chart 1).[20–22]

Keywords: Modified electrodes; Ethylenedioxythiophene; Electrochemical biosensing; Ferrocene.
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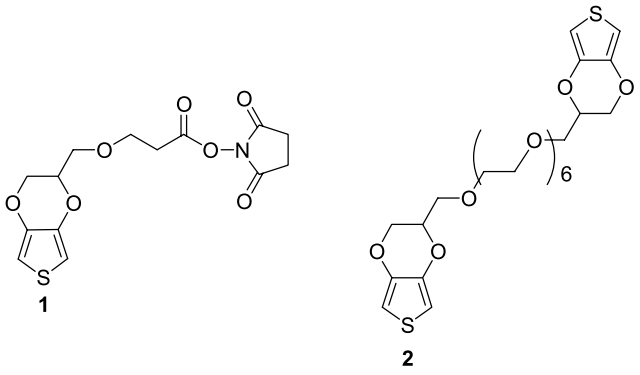
ODN 5.3	5' **Fe-**GTA TTC CTT GGA CTC ATA AGG T-**Fe-**C7-NH_2 3'
ODN 3.3	5' GTA TTC CTT GGA CTC ATA AGG T-**Fe-Fe-**C7-NH_2 3'
ODN 3.3.3	5' GTA TTC CTT GGA CTC ATA AGG T-**Fe-Fe-Fe-**C7-NH_2 3'

Chart 1. List of ferrocene Fc-ODNs used for film preparation.

In this paper, the post-functionalization of a poly(ethylenedioxythiophene) (PEDOT) film with the Fc-ODN systems was performed. So far the PEDOT backbone has not been investigated for the generation of DNA sensors although it represents a preferential conducting polymer for such application owing to its high chemical stability, compatibility with aqueous media, and conductivity.[23] According to our previous results on PEDOT-based modified electrodes,[24] we describe here the synthesis of a novel hydroxysuccinimidyl ester derivative of EDOT, **1**, and the electrochemical copolymerization of **1** and the bis-EDOT compound, **2** (Scheme 1).[25] It is shown that a redox signal corresponding to the ferrocene units appear upon covalent immobilization of ferrocene-ODN assemblies via amide bond formation, which directly confirm, the probes grafting.



Scheme 1. Precursors of copolymer.

2. Experimental

2.1. Reagents

Lithium hydride (LiH), 18-crown-6, ethyl-3-bromopropionate, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, *N*-hydroxysuccinimide were purchased from Aldrich and used as received. THF (anhydrous) and DMF (anhydrous) were purchased from ACROS. Diethylether, absolute ethanol, ethyl acetate, cyclohexane were purchased from CarloErba, silica gel (240–400 mesh) from Merck and $MgSO_4$ from SDS. All aqueous solutions were made with MilliQ purified water (Maxima ELGA). Phosphate buffers (pH=6.8) were made with 0.25 M KH_2PO_4, 0.25 M Na_2HPO_4 and 0.75 M NaCl. 2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-2-yl)methanol (hydoxymethyl-EDOT) and hexaethylene glycol Bis(2,3-dihydrothieno[3,4-*b*][1,4]-dioxin-2-ylmethyl)ether **2** were prepared as described in the literature.[23,25]

2.2. Physical and spectroscopic methods

Melting point are uncorrected and were measured on a Electrothermal 9100 apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer at 250 and 62.5 MHz, respectively. UV spectra were obtained on a Varian Cary 1E spectrophotometer. Mass spectrometry was performed with a JEOL FX 102 Mass spectrometer in the FAB mode (Laboratoire de Mesures Physiques, USTL, Montpellier, France). MALDI-TOF mass spectra of oligonucleotides were recorded at the IBCP, Lyon on a Voyager DE (Perseptive Biosystems, Framingham, MA, USA) mass spectrometer equipped with an N_2 Laser. The matrix used for oligonucleotide mass analyses was hydroxypicolinic acid (HPA). Calibration was carried out using internal data base reference.

2.3. Synthesis of functionalized EDOT

2.3.1. 3-(2,3-Dihydro-thieno[3,4-*b*][1,4]dioxin-2-yl-methoxy)-propionic acid ethyl ester 3. A mixture of NaH (110 mg, 2.73 mmol, 60% in mineral oil) and 18-crown-6 ether (13 mg, 0.05 mmol) in 2.8 mL of anhydrous THF was stirred under argon at −10 °C. 2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-2-yl)methanol (Hydroxymethyl EDOT) (280 mg, 1.63 mmol) in 2.8 mL of anhydrous THF was added. The mixture was stirred at room temperature for 1 h and then cooled down to 0 °C. Ethyl-3-bromopropionate (500 mg, 2.76 mmol) in 2.8 mL of anhydrous THF was added drop by drop during 10 min. After 72 h under stirring at room temperature the mixture was diluted with 25 mL of 5% NH_4Cl aqueous solution. The mixture was extracted with diethylether (3×25 mL). The combined organic phases were washed with water, dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified on a silica gel column using cyclohexane–ethyl acetate (70:30 v/v) as eluent to afford **4** (93 mg, 21%) as a pale yellow oil. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.12 (dd, $^3J=7.2$ Hz and $^3J=7.0$ Hz, 3H, CH_3), 2.44 (dd, $^3J=6.5$ Hz and $^3J=6.2$ Hz, 2H, CH_2–CO), 3.46–3.59 (m, 2H, CH_2O), 3.64 (t, $^3J=6.2$ Hz, 2H, OCH_2 CH_2CO), 3.84–4.19 (m, 5H, CH_3–CH_2O, CH, CH_2–CH), 6.17 (s, 2H, $H_{thiophene}$) ppm. ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 14.2, 35.0, 60.6, 66.0, 67.2, 69.3, 74.5, 99.7, 141.5 (*C*H), 171.3 (C=O). MS (FAB): 273 (M^{+1}, 16), 272 ($M^{\cdot +}$, 12), 89 (16), 55 (11), 29 (2).

2.3.2. 3-(2,3-Dihydro-thieno[3,4-*b*][1,4]dioxin-2-yl-methoxy)-propionic acid 4. Compound **3** (37 mg, 0.14 mmol) in 2 mL of absolute ethanol and 2 mL of an aqueous solution of NaOH (22 mg, 0.54 mmol) were stirred at reflux during 6 h. After the reaction mixture was cooled to room temperature, the solvents were evaporated in vacuo. The crude product was dissolved in 40 mL of water and extracted with diethylether (2×30 mL). The aqueous phase was acidified with HCl 2 M until acidic pH and extracted again with diethylether (2×30 mL). The second combined organic layers were dried over $MgSO_4$, and evaporated in vacuum to afford 33 mg (0.14 mmol, quantitative yield) of a white powder corresponding to compound **4**: mp 76–78 °C; ^1H NMR (270 MHz, C_5D_5N) δ: 4.08 (dd, $^3J=6.0$ Hz and $^3J=6.6$ Hz, CH_2COOH), 4.93–5.07 (m, 2H, CHCH_2O),

5.19 (t, $^3J=6.3$ Hz, 2H, OCH_2CH$_2$COOH), 5.35–5.63 (m, 2H, CH_2CH), 5.64–5.66 (m, 1H, CH), 7.86 (s, 2H, $H_{thiophene}$), 10.0 (s, 1H, OH) ppm. ^{13}C NMR (62.5 MHz, C_5D_5N) δ: 37.8, 68.4, 70.0, 71.6, 75.2, 102.2 (CH), 175.9 (C=O) ppm. MS (FAB): 244 ($M^{\cdot+}$, 50); 89 (21). Anal. Calcd: C, 49.17; H, 4.95. Found: C, 49.33; H, 4.89.

2.3.3. 3-(2,3-Dihydro-thieno[3,4-*b*][1,4]dioxin-2-yl-methoxy)-propionic acid 2,5-dioxo-pyrrolidin-1-yl ester 1. A mixture of **4** (110 mg, 0.45 mmol), 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (121 mg, 0.63 mmol), *N*-hydroxysuccinimide (62 mg, 0.54 mmol) in 2 mL of anhydrous DMF was stirred under argon atmosphere at room temperature during 20 h. After addition of acetone (10 mL) and water (10 mL), the mixture was extracted (3×30 mL) with a mixture of diethylether and ethylacetate (2/1 v/v). The combined organic layers were washed with water, dried over $MgSO_4$, and evaporated in vacuum to afford 154 mg (0.45 mmol, quantitative yield) of **1** as a white solid: mp 84–85 °C; ^{1}H NMR ($CDCl_3$) δ: 2.63 (s, 4H, CH_2 (succi)), 2.70 (dd, $^3J=6.0$ Hz and $^3J=6.3$ Hz, 2H, CH_2CO), 3.52 (m, 2H, CH_2O), 3.69 (t, $^3J=6.0$ Hz, 2H, OCH_2CH$_2$CO), 3.84–4.08 (m, 2H, CH_2CH), 4.10–4.15 (m, 1H, CH), 6.13 (s, 2H, $CH_{thiophene}$) ppm. ^{13}C NMR ($CDCl_3$) δ: 26.4, 33.0, 66.8, 67.1, 70.3, 73.2, 100.4, 142.4, 167.4 (C=O), 169.8 (C=O) ppm. MS (FAB): 341 ($M^{\cdot+}$, 10). HRMS (FAB): calcd for $C_{14}H_{15}O_7NS$ 341.0569, found 341.0564. Anal Calcd: C, 49.26; H, 4.43; O, 32.81; N, 4.10; S, 9.39. Found: C, 50.08; H, 4.98; O, 29.20; N, 3.83; S, 8.08.

2.4. Ferrocenyl-labelled oligonucleotides

Ferrocenyl-labelled oligonucleotides **ODN 5.3** and **3.3** were synthesized as previously described.[20–22] The ODN probes have a terminal amino group at the 3′ position allowing covalent linking on the copolymer film (Chart 1). Oligonucleotide target, complementary to the oligonucleotide probes with 5′-ACC-TTA-TGA-GTC-CAA-GGA-ATA-C-3′ sequence was provided by the bioMerieux company. The noncomplementary oligonucleotide is T20: 3′-TTT-TTT-TTT-TTT-TTT-TTT-T-5′.

ODN 3.3.3 containing free ferrocenyl groups was synthesized using an Applied Biosystems 394 RNA/DNA synthesizer. 1-[3-*O*-dimethoxytritylpropyl]-1′-[3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidityl) propyl]ferrocene[20] was dissolved in anhydrous acetonitrile (C= 0.09 M) and the solution was dried on 3 Å molecular sieves during 24 h. Solution was filtered on 0.22 μm PVDF filter before loading on DNA synthesizer. Standard 1 μmol coupling cycle was used for oligonucleotide elongation. The coupling reaction time was increased from 15 to 500 s for ferrocene synthons. After ODN synthesis (DMTr ON), controlled pore glass (CPG) support was treated in NH_4OH (30% aqueous) during 16 h at 55 °C, then supernatant was recovered and evaporated to dryness under vacuum. The pellet was dissolved in 500 μL of MilliQ water plus 500 μL of MOP buffer. ODN was purified on MOP column (CTGen, San Jose, CA) as followed: column was first washed with 2 mL of CH_3CN/H_2O (1:1) and 2 mL of TEAAc buffer 0.1 M (pH=7) successively before loading the ODN solution on column. Truncated sequences were eluted with 4 mL of TEAAc buffer 0.1 M (pH=7). ODN was eluted with 1 mL of CH_3CN/H_2O (1:1) and solvents were evaporated to dryness. Then, ODN was detritylated with 300 μL of 80% acetic acid/water during 30 min. Acetic acid was eliminated by evaporation and ODN was precipitated twice with 0.3 M AcONa (300 μL) and ethanol (900 μL) before lyophilization in water. Oligonucleotide was purified by HPLC on a RP-18e Lichrospher 100 (300×7.5, 10 μ, Merck) with linear gradient of acetonitrile (5–40%) in 0.05 M aqueous triethylammonium acetate (pH=7). HPLC analyses of ODN purities was run on a reverse-phase RP-18e Chromolith Performance column (100×4.6 mm, Merck) with the same eluents than above described. ODN 3.3.3 was characterized by MALDI-TOF mass spectral analyses. The result (*m*/*z* calcd 8039.05, found 8042,40.70) illustrate the successful incorporation of ferrocenyl moieties into the oligonucleotide. The difference between calculated and found masses is attributed to the calibration of the instrument with standards with a behavior similar to ferrocenyl-ODN. The same problem was observed in literature.[22,26]

2.5. Electrochemical materials

Cyclic voltammetry data were acquired using a computer-based Bioanalytical instrument (BAS 100) electrochemical workstation with a three-electrode setup. A 1.6 mm diameter gold working electrode was used and polished with 1–0.1 μm diamond paste and ultrasonically rinsed in absolute ethanol. Counter electrode in platinum and Ag/AgCl (with 3 M NaCl filling solution) reference electrode were used. All anhydrous solvent are of electronic grade purity.

2.6. Electrochemical polymerization

The copolymer was obtained from electropolymerization of precursor **1** and **2** in acetonitrile containing 10^{-1} M nBu$_4$NCF$_3$SO$_3$ as supporting electrolyte at a fixed potential of 1.3 V/Ag/AgCl. Therefore, their initial concentration were kept the same: 0.01 M. 5 mC cm^{-2} were deposited. Acetonitrile with 10^{-1} M nBu$_4$NCF$_3$SO$_3$ was used to analyse the co-polymers. Cyclic voltammograms were recorded without ohmic drop compensation and at a scan rate of 100 mV/s. All cyclic voltammetry experiments were carried out at 25 °C using a cell equipped with a jacket allowing circulation of water from the thermostat.

2.7. Covalent immobilization of the ferrocene-ODN probes onto PEDOT

Substitution of the succinimidyl ester groups of the copolymer using the amino Fc-ODN probes was carried out by simple immersion of the modified electrode during 3 h at 25 °C in acetonitrile/phosphate buffer pH 6.8 (80/20 v/v) solution of the Fc-ODN probe (6.25 μM). To remove any ungrafted Fc-ODN probes, the modified electrode was thoroughly washed with water. The grafted modified electrode was then analysed by cyclic voltammetry in acetonitrile solution containing 10^{-1} M nBu$_4$NCF$_3$SO$_3$ as supporting electrolyte.

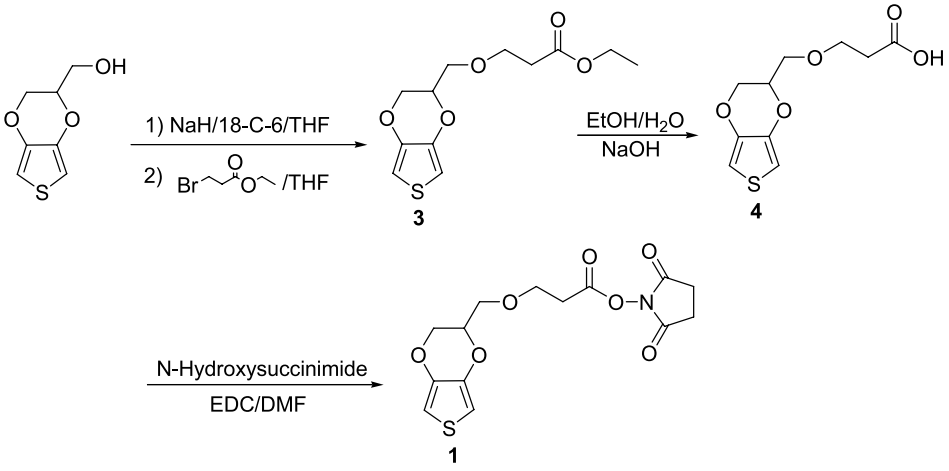
2.8. Hybridization of ODN target

PEDOT-ODN 5.3, **3.3** and **3.3.3** modified electrodes were incubated at 37 °C during 3 h in a phosphate buffer (pH 7.4) containing the ODN target (6.25 μM). After incubation, the modified electrodes were rinsed with the same buffer and electrochemically characterized in acetonitrile solution containing 10^{-1} M nBu$_4$NCF$_3$SO$_3$ as supporting electrolyte.

3. Results and discussion

3.1. Synthesis of the functionalized EDOT monomer 1

The synthetic route toward **1** is outlined in Scheme 2. The ester **3** was synthesized in 21% yield from hydroxymethyl EDOT by a Williamson reaction with ethyl-3-bromopropionate. The hydrolysis of ester group was performed in presence of NaOH to give quantitatively the carboxylic acid **4**. Reaction of **4** with *N*-hydroxysuccinimide in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride reagent lead quantitatively to the precursor **1**.



Scheme 2. Synthesis of precursor **1**.

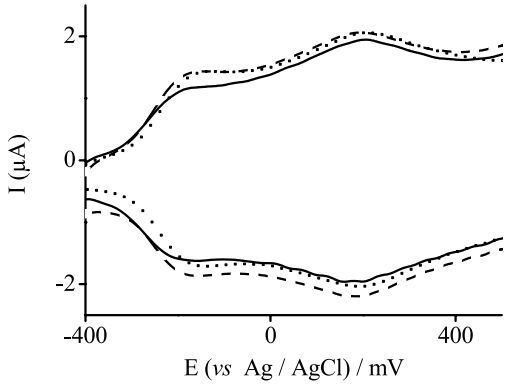


Figure 1. Cyclic voltammogram of copolymer in 0.1 M nBu$_4$NCF$_3$SO$_3$/CH$_3$CN, scan rate 100 mV s^{-1}. (Film grown under potentiostatic conditions, see text). Before immersion in solutions (dotted line); after immersion in grafting solution (dashed line); after immersion in grafting and hybridization solutions (solid line).

3.2. Co-electropolymerization of precursors

The precursor copolymer was formed easily on the gold electrode by electropolymerization of monomers **1** and **2** (10^{-2} M each) in an acetonitrile solution of nBu$_4$NCF$_3$SO$_3$ (10^{-1} M). Monomers **1** and **2** were shown to polymerize at the same oxidation potential.[24] The 5 mC cm^{-2} film was grown in potentiostatic conditions at 1.3 V/Ag/AgCl. The peak current of the electrochemical response of copolymer in acetonitrile was found to increase linearly with the scan rate, as expected for a confined surface electrochemical process with the formation of a stable adhesive film (Fig. 1). The cyclic voltammogram (CV) of the resulting co-polymer shows two anodic waves around −0.16 and +0.25 V characteristic of the PEDOT backbone. In order to test the influence of different solutions on the co-polymer, it was immersed 3 h at 25 °C in acetonitrile/phosphate buffer solution without ODN probes then washed and immersed 3 h at 37 °C in phosphate buffer without ODN target. The CV before and after immersion are similar. A little decrease of the current intensity is observed for the two redox systems of the PEDOT after the second immersion with a 40 mV negative shift for the first oxidation peak. This behavior reflects the change in solvation of the co-polymer when exposed to different solutions. Repetitive scans did not induce any change in the CV curves, which indicated a good stability of the PEDOT film.

3.3. Covalent immobilization of the ferrocene-ODN probes

After covalent grafting of the Fc-ODN probes, a small decrease of the current intensity of the first redox system of the copolymer was observed and attributed to the solution effects (Fig. 2 and Table 1). In contrast no decrease of the current intensity was observed for the second redox system of the copolymer except for **ODN 3.3.3**. In all cases a new redox system corresponding to the oxidation and subsequent reduction of ferrocenyl group was observed. The current intensity of the ferrocene redox system was observed to be depending on the nature of the ODN probe.

With **ODN 5.3** grafted onto co-polymer the anodic shoulder

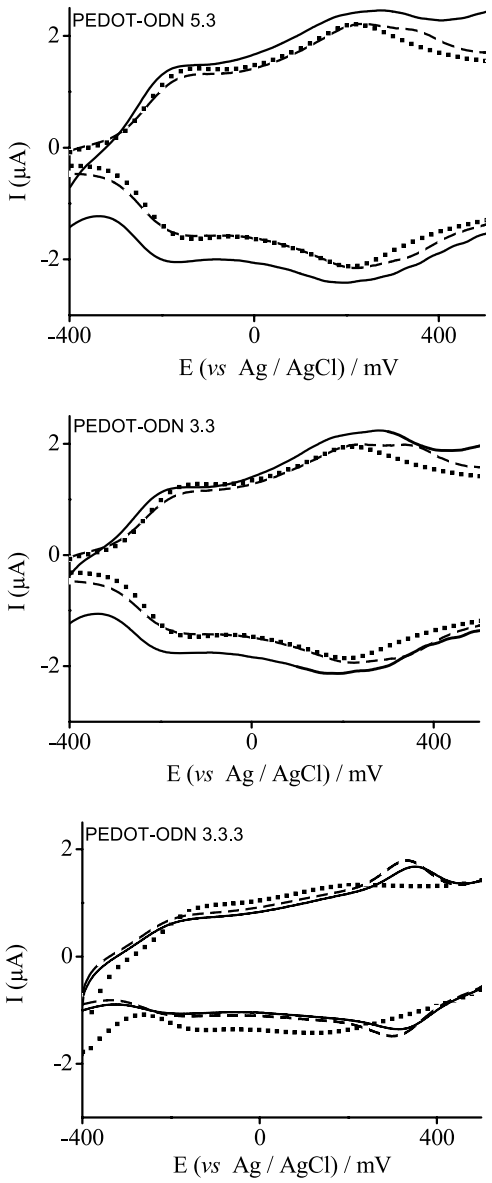


Figure 2. Cyclic voltammograms between −400 and 500 mV of functionalized copolymers in 0.1 M nBu$_4$NCF$_3$SO$_3$/CH$_3$CN, scan rate 100 mV s^{-1}. Before grafting (dotted line) and after grafting with a Fc-ODN (dashed line); after hybridization with target ODN (solid line).

associated with the ferrocene system was observed at ca. 0.3–0.4 V. The CV of co-polymers grafted with **ODN 3.3** show that the anodic shoulder associated with the ferrocene system become an anodic wave accompanied by the emergence of the subsequent cathodic shoulder. The most important effect is obtained with **PEDOT-ODN 3.3.3**. The CV curve of **PEDOT-ODN 3.3.3** exhibit the characteristic peaks of ferrocenyl groups with a quasi-ideal redox system, the oxidation and reduction peaks being respectively observed at 0.33 and 0.32 V.

The use of Fc-ODN probes allow direct assessment of the linkage of the ODN probes on the polymer film by visualization of the ferrocenyl electrochemical response that increases with the number of ferrocene units. Moreover, by exact integration of the areas under the ferrocene anodic peaks corrected from background current the efficiency of the post-functionalization of the co-polymer can be estimated. The exact integration of the amount of charge exchanged by ferrocenyl groups during the CV for **PEDOT-ODN 3.3.3** corrected from background current corresponded to an electrode coverage of 78 pmol cm^{-2}.

3.4. Incubation with non-complementary DNA strand

The PEDOT-Fc-ODN modified electrode were incubated at 37 °C during 3 h in a phosphate buffer (pH 7.4) containing the non-complementary ODN target (6.25 μM). After incubation, the modified electrode was rinsed with the same buffer solution and electrochemically characterized in acetonitrile. In all cases the electrochemical response of the modified electrodes before and after incubation with non-complementary ODN target were identical (Fig. 3).

3.5. Hybridization with ODN target

The hybridization of **PEDOT-ODN 5.3** and **3.3** with the complementary ODN target led to an increase of the current intensity and a 60 mV negative shift for all oxidation waves. The oxidation peaks of the ferrocenyl group shift from 0.35 to 0.29 and from 0.36 to 0.30 V for **PEDOT-ODN 5.3** and **3.3**, respectively. The negative shift of potentials can be attributed to a faster electron transfer through the copolymer after hybridization. Pharm et al. interpreted the current enhancement upon hybridization on the basis of changes in the conformation of the ODN strands. Indeed, single-stranded ODNs behave as random coils while after hybridization of a complementary sequence the double-stranded ODNs lead to a more organized surface, through which counter ions could diffuse more freely.[9] Additionally it is believed that the hydrophilic character of hybridization of DNA combined to the highly hydrophilic character of EDOT-based polymers containing oligooxyethylene chains can increase the permeability of the polymer films to doping anions. With **PEDOT-ODN 3.3.3** the hybridization induces a decrease in the current density together with a positive shift of the oxidation wave, as generally observed with conjugated polyheterocycles functionalized with ODN recognition centres. The decrease of the current intensity and a positive shift of oxidation potential peak were explained by a decrease of the permeability of the polymer

Table 1. Electrochemical values for the grafting of ODN-Fc and hybridization with complementary DNA target

ODN entry	Probe surface density corrected from background (pmol cm^{-2})	Variation of peak height of ferrocene oxidation wave after hybridization corrected from background grafting curve (μA)
ODN 5.3	32 (12)	0.37 (0.05)
ODN 3.3	46 (10)	0.27 (0.05)
ODN 3.3.3	78 (8)	−0.10 (0.03)

The values in parentheses represent the standard deviations from several electrode measurements ($n>4$) not the nonlinear curve fitting errors.

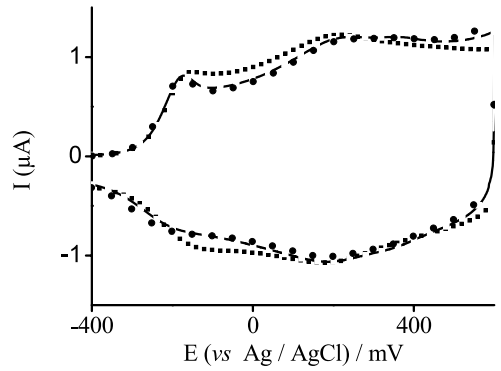


Figure 3. Cyclic voltammogram of **PEDOT-ODN 3.3.3** between −400 and 600 mV in 0.1 M nBu$_4$NCF$_3$SO$_3$/CH$_3$CN, scan rate 100 mV s^{-1}. Before grafting (dotted line), after grafting Fc-ODN (dashed line) and after incubation with non-complementary ODN target (circle dashed line).

films to doping anions and to the change of conformation of the conjugated backbone.[11–16]

These results are very interesting for the development of direct DNA hybridization electrochemical sensors as, to our knowledge, there is no reported example in the literature describing an increase of the current intensity after ODN probes linkage followed by a new enhancement of current intensity after hybridization of the ODN target. Moreover, this strategy allows the determination of the optimal position and the number of the ferrocenyl groups into the ODN probe sequences.

4. Conclusion

A succinimidyl ester derivative of EDOT has been synthesized and characterized. Its co-electropolymerization with oligo(oxyethylene)diEDOT in potentiostatic conditions in acetonitrile leads to a stable film. The use of this new copolymer film for the immobilization of ferrocene-modified ODN probes shows a sensitive and selective DNA sensor. Indeed hybridization of ODN target is observed to induce a positive shift of the oxidation waves and an increase of the current intensity. The latter increase is more important when three ferrocenyl groups are near the electrode surface. These results shows great promise for the development of direct DNA hybridization electrochemical sensors, as to the best of our knowledge, there are so far no examples reported in literature which describes an increase of the current intensity after ODN probes immobilization followed by a new enhancement of current intensity after hybridization of the ODN target. Moreover, this work also emphasizes the subtle balance between the number and position of the ferrocenyl groups in ODN probe sequence in order to achieve accurate electrochemical sensing of DNA hybridization.

Lastly this works validate ours simple strategy to prepare labelled ODN with one or more electroactive markers at different positions of the chain[22] because it is necessary to test different combination of Fc-ODNs to obtain the best DNA sensors. Work to prepare ODN probes with two and three ferrocenyl groups in 3′ position with one and more 5′ ferrocenyl groups is currently underway.

Acknowledgements

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