



The effect of the oxidation state of a terthiophene-conducting polymer and of the presence of a redox probe on its gene-sensing properties

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ABSTRACT

The gene-sensing properties of sensor films made of a terthiophene-conducting polymer, poly(3-((2':2'', 5'':2''-terthiophene)-3''-yl)acrylic acid) (PTAA), were evaluated using electrochemical impedance spectroscopy for films in their reduced and oxidised states with and without the $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probe (RP) in dilute tris-EDTA buffer. Porous films of PTAA were prepared and attached to an oligonucleotide sequence specific to the *Salmonella* virulence gene *InvA*. These films could be described with a dual transmission line model in which the polymer conductivity was increased as a consequence of surface binding of complementary DNA. The effect is analogous to that reported for silicon nanowires and field-effect transistors in dilute electrolyte modified by charge exchange across the polymer-electrolyte interface. As a result, gene sensing could be conveniently observed as a change in the impedance phase angle at a fixed frequency.

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1. Introduction

Conducting polymers (CPs) hold promise to be effective materials for designing sensors that can detect genes without the cost inherent in detection techniques requiring fluorescent DNA (Dore et al., 2006). In that regard, label-free detection sensors are being developed that use electrochemical impedance spectroscopy (EIS) (Bonanni et al., 2005). Oxidation of CPs can create self-localized excitons (polarons and bipolarons) in the material that are associated with simultaneous dopant diffusion into the polymer (MacDiarmid, 2002). A question that arises when using EIS with CPs is whether the film possesses better sensing properties when used in an oxidised or reduced state. Recently we have reported synthesis of poly(3-((2':2'', 5'':2''-terthiophene)-3''-yl)acrylic acid) (PTAA) and demonstrated its use in DNA sensing (Peng et al., 2007d).

In addition to the oxidation state of the CP film, another question about EIS on CPs concerns the use of a redox probe during the experiment. The $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probe (RP) has been widely used to facilitate a measurement of the charge transfer resistance (R_{ct}) (Peng et al., 2007c). However, for those sensors where a measurable R_{ct} cannot be obtained another technique

for performance evaluation is required. An alternative is to select one frequency from the impedance data and measure the performance at that frequency rather than over a range of frequencies (Peng et al., 2006). An advantage of this technique is the ability to measure the performance of a sensor regardless of its conformity to an idealised Nyquist plot. Another advantage is the elimination of the errors associated with estimating the fitting R_{ct} parameters. Care must be taken however to choose a frequency that fairly represents the sensor hybridization performance. A phase angle Bode plot has been used to identify 50 Hz and 5000 Hz as two frequencies where the hybridization response occurred in a thiophene sensor functionalized with an arylsulfonamide group (Gautier et al., 2007b). The same authors (Gautier et al., 2007b) also compared the sensor response with and without $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probe in order to compare the hybridization event observed by Faradaic and non-Faradaic processes.

In the present paper, the gene-sensing properties of PTAA sensor films were evaluated using EIS with and without the $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probe in dilute tris-EDTA buffer. Films were rendered in either their reduced state by applying a 200 mV constant bias potential or in their oxidised state by applying a 900 mV constant bias potential. The experiments were designed to answer the question of whether gene sensing was enhanced or hindered using the redox probe and having the polymer in either the oxidised or reduced state.

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2. Experimental

2.1. Reagents

The monomer 3-((2'-2'', 5''-2'''-terthiophene)-3''-yl) acrylic acid (TAA) was synthesized as previously reported (Peng et al., 2007d). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), tetrabutylammonium trifluoromethanesulfonate (TBACF₃SO₃), acetonitrile (CH₃CN) and PBS pellets were purchased from Sigma–Aldrich. EDTA (ethylenediaminetetraacetic acid), disodium salt, dihydrate was purchased from Scharlau. Potassium ferrocyanide (K₄Fe(CN)₆·3H₂O) was purchased from J.T. Baker Chemical Company. Potassium ferricyanide (K₃Fe(CN)₆) was purchased from The British Drug Houses Ltd. Tris(hydroxymethyl)methylamine (tris) was purchased from AppliChem.

Oligonucleotides (ODN) were synthesized by Invitrogen Life Technologies or Alpha DNA. The ODN probe was end-modified to bear a terminal amino group on its 5'-end with the sequence 5'-NH₂-GATGAGTATTGATGCCGA-3'. The probe sequence is specific to the *Salmonella* virulence gene *invA* (Galan et al., 1992). The complementary ODN sequence was 5'-TCGGCATCAATACTCATC-3'. ODN solutions were prepared with 10 mM tris:1 mM EDTA solution (pH 8.0). Tris–HCl and EDTA solutions were prepared with Milli-Q water.

2.2. Electropolymerization of the monomer

Electropolymerization of the TAA monomer to form the PTAA polymer film was performed at room temperature using a CHI 440 electrochemical workstation (CH Instruments). A three-electrode cell was used containing a 3.0-mm glassy carbon (GC) working electrode (Bioanalytical Systems, BAS), a Pt counter electrode (BAS), a Ag/AgCl (in saturated KCl) reference electrode, a 5.0 mL TAA monomer solution, 50 mM TBACF₃SO₃ as electrolyte and CH₃CN as solvent. Prior to electropolymerization the working electrode was polished on 0.5- μ m alumina slurry mixed with Milli-Q water. After polishing the electrode was immersed in solutions of acetonitrile containing activated carbon, CHCl₃, and Milli-Q water and sonicated for 10 min. Prior to electropolymerization, the electrolyte solution was deaerated by purging with nitrogen. The charge applied for electropolymerization using constant current (CC) mode was held at 0.7 mC by applying 0.1 mA for 7 s. After the films were synthesized they were rinsed with acetonitrile and PBS buffer.

2.3. AFM measurements

Samples for atomic force microscopy (AFM) were prepared as above with the addition of a tape mask on one half of the glassy carbon electrode. A step edge was created by peeling the tape following deposition. AFM scans were taken using NSC16 probes (Mikromasch) and an 80- μ m range, closed-loop scanner on a Q-Scope Q250 scanning probe microscope (Ambios Instruments) running in the tapping mode. Scans were taken with a range of 80 μ m and a resolution of 500 \times 500 at a scan rate of 8 μ m s⁻¹. AFM data was analysed in Ambios Q-Port SPM analysis software (v.1) following acquisition. For step height measurements a representative average baseline height of the glassy carbon region was subtracted from a representative average thickness of the polymer film across five scan lines. Average and root mean square (RMS) roughness near the step was calculated for approximately 20 μ m \times 20 μ m regions on the glassy carbon electrode.

2.4. Pre-probe attachment potential treatment

Before the probes were attached the films were pre-treated with a constant bias potential of either 200 mV or 900 mV for 60 s in 10 mM tris–1 mM EDTA buffer.

2.5. Covalent attachment of ODN probes onto the polymer film

After applying the constant potential treatment, ODN probes were covalently attached onto the PTAA film according to a previously described procedure by adding 10 μ L of 434 μ M probe to the PTAA film followed by 40 μ L of EDC and allowing attachment to occur for 1 h at 28 °C (Walsh et al., 2001). Fresh 40-mM EDC solution was prepared by adding a quantity of EDC to a calculated volume of PBS buffer (pH 5.2).

2.6. Hybridization

0.136 nM, 13.6 nM, 1.35 μ M, and 1.36 μ M complementary (com) target ODN were allowed to hybridize for 1 h at 37 °C then rinsed with tris–EDTA buffer.

2.7. Electrochemical impedance characterization of probe immobilization and hybridization

Electrochemical impedance spectra were recorded on an EG&G potentiostat/galvanostat (Princeton Applied Research, model 280) coupled to an EG&G 1025 Frequency Response Analyzer in 10 mM tris–1 mM EDTA solution. A three-electrode cell with a volume of 6 mL containing the glassy carbon working electrode, a Pt counter electrode and Ag/AgCl (in 3 M KCl) reference electrode was used. Impedance was performed with 5 mV sinusoidal modulation amplitude at an applied bias potential of either 200 mV or 900 mV vs. Ag/AgCl (in 3 M KCl) over the frequency range from 10 Hz to 10⁵ Hz with or without the 5 mM Fe(CN)₆^{3-/4-} redox probe. The redox probe was made from tris–EDTA buffer pH (8.0) and 1:1 molar ratio of K₄Fe(CN)₆·3H₂O/K₃Fe(CN)₆.

3. Results

Film thickness and topology for PTAA films electrodeposited on glassy carbon electrodes was evaluated by atomic force microscopy. A typical film, such as shown in Fig. 1, had a thickness of 566 \pm 65 nm as determined from step heights created using a tape mask and peel. The bulk of the film was of a globular morphology with multiple full-thickness aggregates comprised of smaller, more uniform particles. Representative average roughness was calculated to be 5.1 nm on the glassy carbon surface, 94.4 nm near the edge and 104.0 nm in the bulk. RMS roughness was comparable at 6.76 nm, 115.0 nm, and 135 nm, respectively. Many of the gaps between globules extended nearly through the full thickness of the film as seen in the line plot in Fig. 1C. This is typical of a conducting polymer formed by diffusion-limited aggregation and the result is a highly porous film with, in this case, pore-sizes of the same order as the thickness.

Given that oxidation of PTAA film begins at an applied potential of about 700 mV (vs. Ag/AgCl) as reported previously (Peng et al., 2007d), the PTAA films synthesized by the constant current method reached a final voltage of about 1.5 V during synthesis resulting in p-doped film with the incorporated anion (CF₃SO₃⁻) that counter-balanced the positive charges on the polymer chains. It has been shown that the CF₃SO₃⁻ anions cannot easily diffuse out of PTAA film prepared in acetonitrile during post-polymerization cycling in PBS solution but rather cations diffuse in and out of

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