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Electrochemistry Communications 8 (2006) 317–322
electrochemistry communications
www.elsevier.com/locate/elecom

Oligonucleotide–functionalised poly(3,4-ethylenedioxythiophene)-coated microelectrodes which show selective electrochemical response to hybridisation

Fouzi Mouffouk, Simon J. Higgins *

Department of Chemistry, University of Liverpool, Crown Street, Liverpool L69 7ZD, United Kingdom

Received 9 November 2005; received in revised form 28 November 2005; accepted 30 November 2005
Available online 18 January 2006

Abstract

A carboxylic acid–functionalised 3,4-ethylenedioxythiophene (EDOT) derivative has been synthesised. Its co-polymerisation with EDOT on a Pt disk microelectrode gave a redox-active poly-3,4-ethylenedioxythiophene (PEDOT) with pendant –COOH groups. These were coupled in situ to an aminoalkyl-terminated oligonucleotide. The p-doping peak in the cyclic voltammogram of the PEDOT changed slightly upon grafting, but underwent a much more marked change upon hybridisation with the complementary oligonucleotide.
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Keywords: Polythiophene; Electropolymerization; Switching; Pendant group; Biosensors; Voltammetry

1. Introduction

Poly(3,4-ethylenedioxythiophene) (PEDOT) has properties which are unique for a polythiophene derivative. It combines great stability in its oxidised state and compatibility with aqueous electrolytes. These properties have led to PEDOT being of interest as a fuel cell membrane material, an electrostatic protection coating, a supercapacitor material, and a biosensor component [1–3]. It is also of great interest as a porous conducting 'scaffold' for covalently-attached redox-active groups [4,5], and for electrostatically-entrapped biomolecules. For example, horseradish peroxidase has been entrapped into PEDOT poly(styrene sulfonate) hydrogels, together with an osmium complex as redox mediator [6]. In a particularly interesting paper, the growth of PEDOT microtubules in the pores of a polycarbonate membrane, in the presence of single-stranded DNA oligonucleotides, was described; the resistance and redox activity of the polymer both diminished when it was exposed to complementary oligos [7]. The stability of PEDOT polymerised in track-etched membrane pores for sensing purposes was found to be greater than that of polypyrrole, which has traditionally been used as a water-compatible conjugated polymer 'scaffold' in sensor applications [8], and a PEDOT-based glucose biosensor based on this technology has been described [9].

Recently, there has been much interest in new technologies for DNA detection. Electrochemical methods offer several advantages here, including relatively cheap instrumentation, easy sample preparation, high sensitivity and comparative simplicity, the latter arising because hybridisation gives rise directly to an electrical signal. Various approaches have been investigated. For example, DNA bases themselves are electroactive and can be irreversibly oxidised electrochemically [10]. Alternatively, specific bases can be oxidised by an electrogenerated mediator such as $[Ru(bipy)_3]^{3+}$ [11]. The problem with the latter approaches is to distinguish between hybridised double-stranded oligonucleotides, and remaining unhybridised probe molecules. Various amplification and labelling strategies have been developed to address these issues [12].

* Corresponding author. Tel.: +44 151 794 3512.
E-mail address: shiggins@liv.ac.uk (S.J. Higgins).

1388-2481/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.elecom.2005.11.027

The incorporation of the probe oligonucleotide into a conjugated polymer is attractive because the electrochemical and electronic properties of conjugated polymers are a sensitive function of their environment, so that hybridisation of the incorporated probes would be expected to perturb the electrochemical response of the polymer. One approach is simply to trap the probe oligo electrostatically within a growing (and therefore positively-charged) polymer film, but this is rather inefficient, and there is no certainty that the trapped oligos will be accessible to the target [7,13]. Alternatively, the oligonucleotide probe can be grafted covalently to the polymer. In an early example of such a study, an activated ester derivative of (1-*H*-pyrrol-3-yl)-acetic acid was electropolymerised, and a 5′-aminoalkyl-terminated oligonucleotide was subsequently coupled to the resulting electroactive polypyrrole [14,15]. Cyclic voltammetry showed that the polymer p-doping peak shifted positive, and diminished, upon hybridisation. The response was larger for thinner films, suggesting that the probe oligos were mostly localised on the outside of the film [15]. The best sensitivity, achieved with the thinnest films, corresponded to about 10^{-12} M target. In subsequent work [16], the sensitivity has been improved by a factor of 10 using the electrochemistry of polymer-anchored ferrocene units, rather than that of the polymer itself, but the multi-step synthesis of the necessary ferrocene-bearing pyrrole monomer is tedious. Interestingly, all of the polypyrrole work so far described has been conducted using macroelectrodes (0.7 cm^2 Pt). One way to boost the sensitivity might be to use microelectrodes, providing that uniform, electroactive films can be grown on such electrodes.

We recently showed that a biotin–functionalised PEDOT derivative could be electropolymerised on a 10 μm Pt disk, to give a polymer-modified electrode that responded to 10^{-16} moles of avidin [17], a significantly lower limit than we had achieved for a related polythiophene system on a macroelectrode [18] We were interested to see whether we could extend this to DNA oligonucleotide detection, by electropolymerising a carboxylic acid–functionalised EDOT derivative and subsequently grafting a single-stranded DNA oligonucleotide probe to the polymer. We report our preliminary results here.

2. Experimental

2.1. Synthesis of *1* [19]

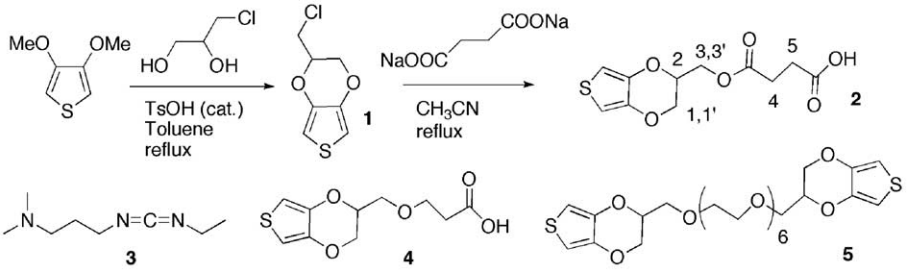
To a mixture of 3,4-dimethoxythiophene (2.00 g, 0.014 mol) and 3-chloro-1,2-propanediol (1.5 g, 0.014 mol) in 'wet' toluene (20 cm^3) was added a catalytic amount of *p*-toluenesulfonic acid and 50 μm water, and the mixture was refluxed for 16 h. The solvent was removed in vacuo, and the residue was taken up in dichloromethane (30 cm^3), washed with water (3×10 cm^3), dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (hexanes) as a pale yellow oil. Yield 1.47 g, 55%. ^{1}H NMR (400 MHz, $CDCl_3$; see Scheme 1 for numbering) δ ppm: 6.35 (m, 2H, thiophene α-H), 4.4–4.0 (overlapping m, 3H,$H^{1,1'}$, H^2), 3.63 (t, 2H, $H^{3,3'}$). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ ppm: 142.0 (thiophene β-C), 100.0 (thiophene α-C), 73.0 (C^2), 72.0 (C^1), 42.6 (C^3). Mass spectrum (EI): [M+H] = 191.

2.2. Synthesis of *2*

Sodium succinate (5.0 g, 31 mmol) was suspended in dry acetonitrile (40 cm^3). To this was added **1** (1.00 g, 5.2 mmol). The reaction was stirred vigorously for 48 h under reflux. Solvent was evaporated, and the residue was partitioned between water and CH_2Cl_2 (30 cm^3 each). The organic extract was dried over $MgSO_4$, filtered and the solvent removed to yield a dark brown oil. This was purified by column chromatography (CH_2Cl_2) to yield **2** (0.71 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm (*J* Hz): 6.35 (m, 2H, thiophene α-H), 4.4–4.0 (overlapping m, 5H, $H^{1,1'}$, H^2, $H^{3,3'}$), 2.72–2.60 (m, 4H, H^4, H^5). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ ppm: 171.9, 168.2 (C═O), 142.0 (thiophene β-C), 100.0 (thiophene α-C), 73.0, 72.5, 72 (C^1, C^2, C^3), 28.9, 28.8 (succinyl –CH_2). Mass spectrum (ES+): [M + H] = 273.

2.3. Electrochemistry experiments

Tetrabutylammonium tetrafluoroborate was recrystallised and dried under high vacuum for several hours prior to use. CH_2Cl_2 was distilled from CaH_2 under Ar immediately before use. Solvent and electrolyte were handled



Scheme 1. Synthesis of **2**, structures of coupling reagent **3** and monomers **4** and **5**.

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