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Specific recognition of a nucleobase-functionalized poly-(3,4-ethylenedioxithiophene) (PEDOT) in aqueous media

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ABSTRACT

In this Letter we report the synthesis, characterization, and electrochemical investigation of a 3,4-ethylenedioxythiophene (EDOT) derivative covalently linked to the nucleobase uracil. The successful electrochemical polymerization of this derivative has provided modified electrodes with a novel functional poly(3,4-ethylenedioxythiophene) derivative. Recognition experiments in aqueous media have shown the specific recognition of the complementary base adenine. The electrochemical detection of the selective binding of nucleobases to this PEDOT derivative in aqueous media can be of particular interest for electrochemical sensor applications in physiological media.

Poly(3,4-ethylenedioxythiophene) (PEDOT) has received much attention owing to its high electrical conductivity, chemical stability, good film forming ability, low band gap, outstanding environmental stability and compatibility with aqueous media.[1,2] Although conducting polymer-based electrochemical sensor devices have emerged as promising tools due to their high sensitivity, easy implementation, low production cost, and ability to miniaturization[3] and in spite of the unique properties of PEDOT among conducting polymers, only recently some efforts were devoted to the development of sensor systems using PEDOT as electronic component or transducer.

Different strategies have been developed to incorporate basic PEDOT in chemical sensors[4–8] including the use of PEDOT nanorods[9] and nanotubes,[10] the fabrication of hydrogel bio-electrodes,[11] the development of molecularly imprinted PEDOT-modified electrodes[5] or simple glucose sensors with micromolar sensitivity based on organic electrochemical transistors.[12] A more general strategy involves the fabrication of PEDOT-based sensors by immobilization of recognition units on polymer thin films which are deposited on metal surfaces.[13–15] Very recently, functionalized PEDOT derivatives have been designed for the use in cation recognition[16] and as sensors.[17,18] Conjugated polymers functionalized with hydrogen bonding receptors have been used to detect complementary biomolecules in analogy to biological systems.[19] Following this strategy, Bäuerle et al. described the electrochemical detection of the selective binding of purine or pyrimidine bases to adenine- or uracil-functionalized poly-(bithiophenes).[20] However, due to the lack of electroactivity of the majority of polythiophenes in aqueous media, the recognition ability of these systems is limited to organic solvents.

In this Letter, we describe the extension of our strategy to systems which are electroactive in aqueous media and which are of particular interest for applications in physiological media. With this goal in mind, we now report the synthesis, characterization, and polymerization of an EDOT derivative **3** covalently linked to the nucleobase uracil. Electrochemical characterization of the polymer in an aqueous electrolyte has clearly shown the influence of the molecular recognition processes on the electronic properties of the uracil-functionalized PEDOT **P3** when the complementary nucleobase is added to the electrolyte.

In the course of our work to develop the synthesis and chemistry of functionalized EDOTs, we recently introduced chloromethyl-substituted EDOT **1** as a novel and convenient intermediate to functionalized PEDOTs.[21] Thus, uracil derivative **3** was obtained by alkylation of uracil (**2**) with chloromethyl-EDOT **1** in the presence of potassium carbonate (Scheme 1). In general, direct N-alkylation of uracil and analogues can yield a N,N′-disubstitution product (**4**), a monosubstituted uracil at the more reactive N-1 position (**3**), and another monosubstituted derivative arising from the alkylation at the N-3 position (**5**) is also possible.[22] In our hands, N-alkylation of uracil (**2**) with chloromethyl-EDOT **1** afforded a mixture from which target N-1-alkylated uracil **3**[23] and N,N'-dialkylated derivative **4**[24] were isolated in 31% and 14% yields, respectively. However, no N-3 alkylated uracil **5** could be isolated from the reaction mixture.

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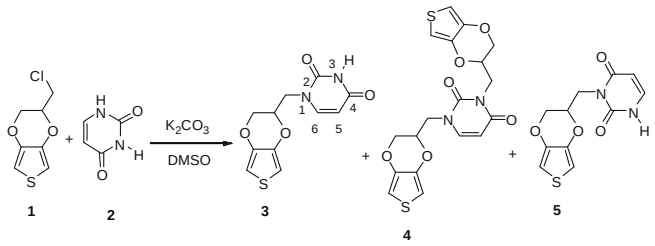

Scheme 1. Synthesis of uracil-EDOT **3**.

Preferent alkylation at the more reactive N-1 position in uracil-EDOT **3** was confirmed by the presence of a singlet at 8.56 ppm in the ^{1}H NMR spectrum as well as by the strong band observed in the FT-IR spectrum at 3187 cm^{-1} both of which are characteristic signals of the free imide group.[25] In order to corroborate substitution at N-1, Nuclear Overhauser Effect (NOE) and Heteronuclear Multiple Bond Correlation (HMBC) experiments were carried out on uracil derivative **3**. As it would be expected from the proposed structures, the NOE experiment indicated an interaction between the olefinic H-6 proton at the uracil ring and the protons of the methylene group adjacent to N-1, evidencing the long-range coupling observed by ^{1}H NMR. In contrast, these methylene protons did not show any remarkable interaction with the further olefinic H-5 proton at the uracil moiety. Additionally, the HMBC experiment revealed a correlation between the carbon of the methylene group and the olefinic H-6 proton at the uracil ring, evidencing the relative proximity between both atoms and thus confirming that alkylation indeed occurred at the N-1 position. The structure and purity of the new EDOT derivatives **3** and **4** were further confirmed by ^{13}C NMR, mass spectrometry, and elemental analysis.

The electrochemical behavior of EDOT-uracil derivative **3** was investigated by cyclic voltammetry (CV) in dichloromethane (DCM) and using tetrabutylammonium hexafluorophosphate (TBAHFP) as the electrolyte. A typical irreversible oxidation wave with a maximum at 0.98 V was measured which is comparable to that of methyl-substituted EDOT (E^{pa} = 1.03 V) under identical conditions (Fig. 1).[26] A clear trace crossing is observable upon oxidation, which points to a nucleation process and fast formation of polymeric material on the working electrode. Thus, in the back sweep of the CV a reduction peak at lower potentials is visible which is due to the reduction of the deposited polymer. Further potentiodynamic polymerization by repetitive scans between −0.75 V and 1.25 V resulted in dark-blue films of uracil-functionalized PEDOT **P3**. The growth of the corresponding conducting polymer film is reflected by the appearance of a novel redox wave at lower potentials than the monomer oxidation which gradually increases in subsequent potential cycles (Fig. 1). The thickness of the electroactive polymer film is steadily increased and can be controlled by the number of cycles. The polymer obtained is strongly adhered to the electrode and provided apparently homogeneous films.

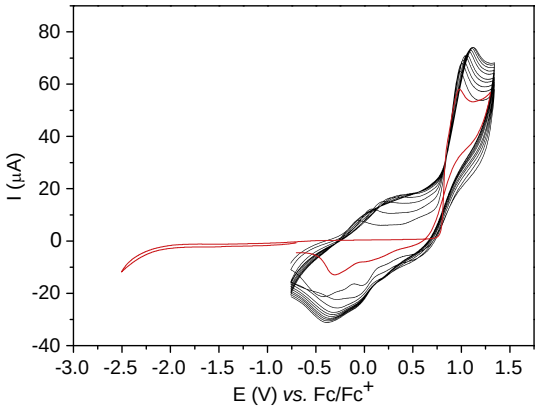

Figure 1. Electrochemical polymerization of EDOT-uracil **3** (black lines) and its characterization (red line) in dichloromethane/TBAHPF (0.1 M). All the measurements were carried out at rt, scan rate of 100 mV/s, Pt disk as working electrode, potentials versus Fc/Fc$^+$.

Uracil-functionalized PEDOT **P3** was electrochemically characterized in an aqueous electrolyte (H_2O/$LiClO_4$, 0.1 M) (Fig. 2a). In the CVs of **P3**, a broad redox wave corresponding to the p-doping/dedoping of the PEDOT backbone is clearly observed. The charging of the conjugated backbone, which is concomitant with the transition from the semiconducting to the conducting state, starts at around E_{lim} = -0.28 V versus Ag/AgCl and the maximum current is found at $E^p_{oxl} = 0.18$ V. A linear variation of the peak currents with the scan rate is observed (insert Fig. 2a) which indicates that the redox active species are anchored to the surface of the electrode. Continuous cycling indicated a high electrochemical stability of **P3** (electroactivity was only reduced to 94% after 100 scans, Fig. 2b) providing an excellent basis for using the polymer in nucleobase recognition experiments.

In order to investigate the effect of specific base pairing onto the electronic properties of the novel uracil-substituted PEDOT **P3**, CVs were taken in the presence of various concentrations of the complementary base adenine **6** (Fig. 3). Despite the uracil moiety is electronically decoupled from the redox-active polymer backbone via the insulated methylene link, the electrochemical response of the PEDOT backbone was strongly influenced by the stepwise addition of adenine **6** in millimolar amounts. A continuous shift of the oxidation peak to more positive potentials, and a shift of the reduction peak to more negative potentials as well as a decrease in overall electroactivity which was determined by the integration of the cyclic voltammograms were observed (Fig. 2c). These effects can be rationalized as a consequence of the formation of hydrogen bonds between the complementary bases at the polymer surface which then form a potential barrier (Fig. 3). During charging of the polymer this potential barrier slows down the diffusion of counterions into the film and thus increases the oxidation potential and reduces electroactivity.[20]

A plot of electroactivity versus the concentrations of added complementary base adenine **6** is represented in Figure 2d. As a comparison and proof for the specific binding, the additions of the non-complementary bases uracil (**2**) and cytosine (**7**) (Fig. 3) to uracil-substituted PEDOT **P3** was also independently investigated. As a result, it is evident that for the complementary base adenine **6** a much bigger effect is found than for the other nucleobases. As a control, basic PEDOT which cannot form hydrogen bonds was prepared and investigated in the presence of the same nucleobases **2**, **6**, and **7**. No significant effect was observed in either case, which unequivocally proofs that in the case of uracil-functionalized PEDOT **P3** the changes in the electrochemical parameters are exclusively due to selective recognition by formation of hydrogen bonds (Fig. 3). It is also worth mentioning that, after recognition of the complementary nucleobase, the modified electrodes do not recover their original electroactivity even after thorough washing.

In summary, we have demonstrated that PEDOT **P3** modified electrodes bearing selective hydrogen-bonding recognition sites can be efficiently fabricated from the novel uracil-functionalized EDOT **3**. The addition of nucleobases in various concentrations led to specific changes in the electrochemical behavior of PEDOT **P3** in aqueous electrolytes. By far the biggest changes in peak potentials and electroactivity were obtained for the complementary base adenine **6**. Furthermore, our results demonstrate that in comparison with analogous rather hydrophobic polythiophenes,

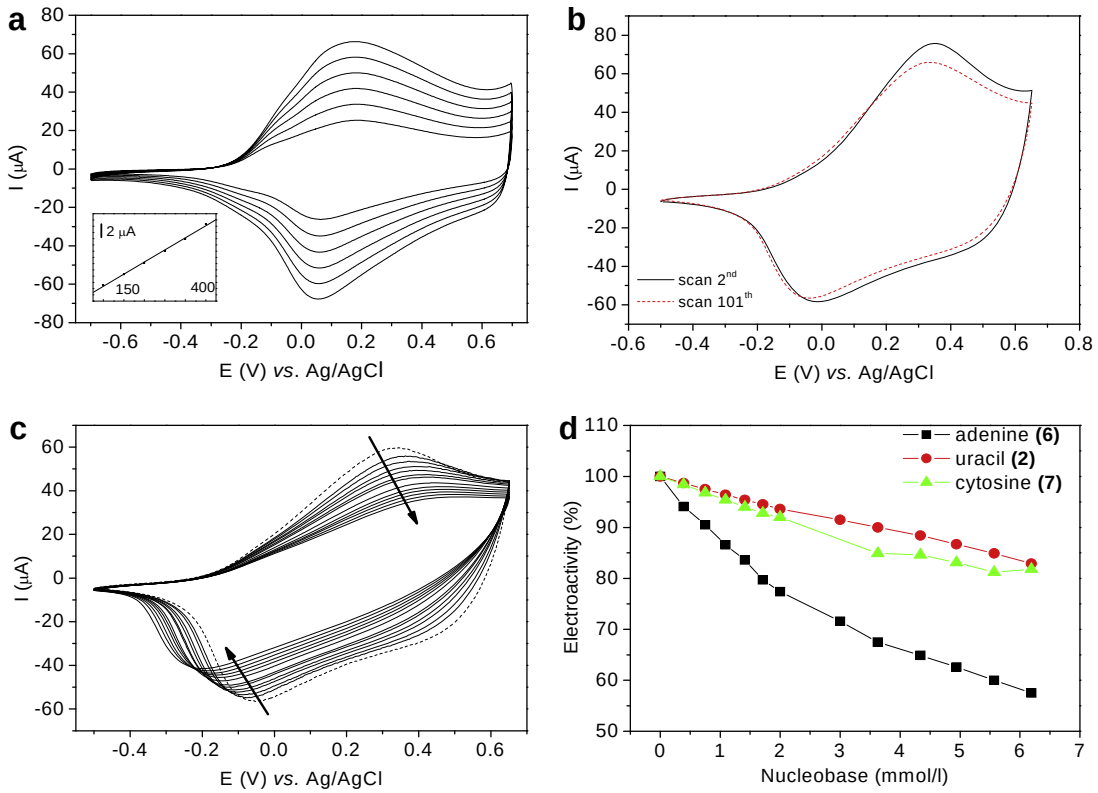

Figure 2. (a) Characterization of uracil-functionalized PEDOT **P3** in $H_2O/LiClO_4$ (0.1 M) at rt, at different scan rates (150, 200, 250, 300, 350, and 400 mV/s), potentials versus Ag/AgCl. Inset: Plot of the peak current versus the scan rate. (b) Decrease in electroactivity after 100 scans in water/$LiClO_4$ (0.1 M) at rt, scan rate of 100 mV/s. (c) Characterization of the uracil-functionalized PEDOT **P3** at a scan rate of 100 mV/s in the presence of adenine (**6**), $c = 0$ (dashed line) up to 6.19 mmol/L, in water/$LiClO_4$ (0.1 M), potentials versus Ag/AgCl. (d) Electroactivity of **P3**, versus the concentrations of added adenine (**6**) (black squares), uracil (**2**) (red circles), and cytosine (**7**) (green triangles).

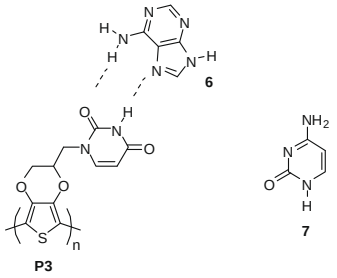

Figure 3. Proposed complexation of the complementary base adenine (**6**) at the polymer surface of the uracil-substituted PEDOT **P3** via hydrogen bonding in water and structure of cytosine (**7**) used in control experiments.

the PEDOT system is superior in terms of water compatibility. These results now pave the way for the incorporation of oligonucleotides into the conducting polymer backbone which should enable the construction of reagentless amperometric oligonucleotide and DNA sensors. The selection of appropriate oligonucleotide fragments to be attached to the EDOT moiety will be important in order to improve selectivity in the detection of complementary oligonucleotides. The water compatibility of the PEDOT systems will allow working in aqueous and physiological media. Work in this direction is currently underway in our groups.

Acknowledgments

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23. ^{1}H NMR ($CDCl_3$, 200 MHz). δ 8.56 (br s, 1H, N-H), 7.16 (d, J = 7.9 Hz, 1H, *Uracil*), 6.39 (AB system, J = 3.7 Hz, 2H, *Th*), 5.71 (dd, J = 7.9 Hz, J = 2.3 Hz, 1H, *Uracil*), 4.53–4.48 (m, 1H, –CH–O–C_{Ar}), 4.28 (dd, J = 12.0 Hz, J = 2.3 Hz, 1H), 4.17 (dd, J = 14.6 Hz, J = 3.2 Hz, 1H), 4.02 (dd, J = 12.0 Hz, J = 5.6 Hz, 1H), 3.84 (dd, J = 14.6 Hz, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz). δ 163.13 (C=O), 150.79 (C=O), 145.27 (N–C=C), 140.80 (C_{Ar}–O), 139.88 (C_{Ar}–O), 102.18, 100.72, 100.61, 71.29, 65.41, 48.48 (CH_2–N); FT-IR (KBr). ν = 3187, 3094, 3058, 1669, 1488, 1454, 1380, 1143, 1018, 858, 783 cm^{-1}; MS (EI) (m/z): 266 (M^+), 154 (M^+–*Uracil*); Anal. Calcd for $C_{11}H_{10}N_2O_4S$: C, 49.62; H, 3.79; N, 10.52; S, 12.04. Found: C, 49.45; H, 3.87; N, 10.44; S, 11.97; mp (methanol): 190 °C.
24. ^{1}H NMR ($CDCl_3$, 200 MHz). δ 7.15 (dd, J = 7.9 Hz, J = 3.6 Hz, 1H, *Uracil*), 6.38 (AB system, J = 3.6 Hz, 2H, *Th*), 6.31 (AB system, J = 3.6 Hz, 2H, *Th*), 5.76 (d, J = 7.9 Hz, 1H, *Uracil*), 4.50–3.80 (m, 10H, –CH–O–); ^{13}C NMR ($CDCl_3$, 75 MHz). δ 162.62 (C=O), 151.70 (C=O), 150.76, 143.54, 143.46, 141.28, 140.83, 101.49, 100.64, 100.54, 99.99, 99.70, 71.04, 71.07, 66.64, 65.45, 49.62 (CH_2–N_1), 40.96 (CH_2–N_3); FT-IR (KBr). ν = 2924, 2854, 1709, 1661, 1483, 1453, 1377, 1139, 1016, 758 cm^{-1}; MS (EI) (m/z): 420 (M^+), 266, 154; Anal. Calcd for $C_{18}H_{16}N_2O_6S_2$: C, 51.42; H, 3.84; N, 6.66; S, 15.25. Found: C, 51.65; H, 3.88; N, 6.44; S, 15.67; mp (dichloromethane/ethyl acetate): 55 °C.
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