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A regioregular polyalkylthiophene bearing covalently-linked biotin, and its interaction with avidin in solution and in thin films†

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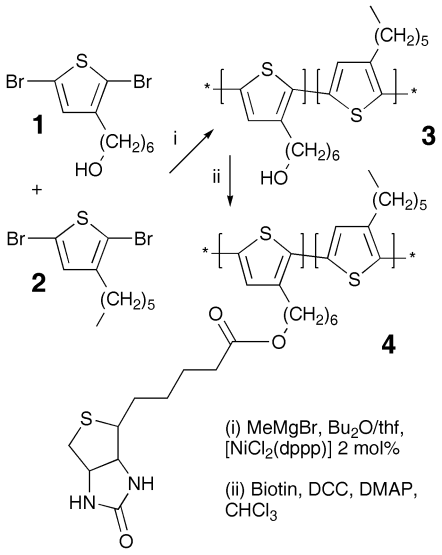
A regioregular copolymer of 3-hexylthiophene and 3-(6-hydroxyhex-1-yl)thiophene has been functionalised with biotin hydrazide; binding of avidin to the biotin moieties causes drastic changes to the absorption spectrum of the polymer in solution, and to the electrochemistry and conductivity of the polymer in thin films.

Polythiophenes bearing covalently-attached receptor sites have properties that make them particularly desirable as sensor materials, and many sophisticated examples are now known.[1–3] Changes in optical, electrical and redox behaviours, induced by binding events at the receptors, are often conveniently detected owing to the comparatively small bandgap, and the modest potentials needed for p-doping.[4,5] Unlike polypyrroles, polythiophenes are stable in both their neutral and p-doped states. Head-to-tail regioregular polyalkylthiophenes[6,7] are of particular interest. They have been shown to have particularly high conductivity when doped, they are highly solvato- and thermochromic,[8,9] and they give unusually high field effect mobility when used as the semiconductor in field effect transistors;[10] this is improved further by doping with electron acceptors.[11] The latter property has been related to the observed self-assembly of the polymers, during solvent casting, into a two-dimensional structure in which the regularly-placed alkyl groups of neighbouring polymer chains are able to intercalate, and efficient π–π stacking between neighbouring polythiophene chains occurs in the remaining dimension, facilitating carrier transport from one chain to another. The synthesis of functionalised versions of these polymers, for sensing and related applications, is therefore of great interest. Examples published to date either involve post-polymerisation functionalization of poly(ω-bromoalkyl)thiophenes,[12] or Stille coupling of appropriate functionalised (and protected) thiophenes or 2,2′-bithiophenes.[13]

Avidin is a highly stable tetrameric glycoprotein (RMM *ca.* 68 kDa), each subunit containing a binding site for biotin; its complex with biotin is exceedingly strong for a noncovalent interaction (K_a 10^{15} M^{-1}).[14–16] Since the early 1970's, the avidin–biotin interaction has been developed for isolation (affinity chromatography), localization (affinity cytochemistry, cell cytometry, and blotting technology), diagnostics (immunoassay, histopathology, and gene probes), hybridoma technology, bioaffinity sensing, affinity targeting, and drug delivery. Sensing of the biotin–avidin binding event is therefore an important topic, and several reports of the use of conjugated polymers in avidin sensing have been published. Among the most elegant examples is the recent use of a biotinylated poly(3-alkyl-4-alkoxy)thiophene to detect avidin binding colorimetrically.[17]

We were intrigued by the possibility of using the unusually favourable optical and electronic properties of regioregular polyalkylthiophenes in biosensing, particularly in thin film form to facilitate solid state device fabrication. We decided to test this idea with the biotin–avidin system, and we have therefore prepared a biotinylated, regioregular polyalkylthiophene using the route outlined in Scheme 1. The [$NiCl_2$(dppp)]-catalysed cross coupling of (6-[iodomagnesio]hexyloxy)trimethylsilane with 3-bromothiophene afforded 6-thiophen-3-ylhexan-1-ol in 63% yield; deprotection of the TMS group occurred during workup. This was dibrominated with NBS to afford **1** in 82% yield. Comonomer **2** was prepared by a literature method.

Rather to our surprise,[18] it proved unnecessary to re-protect monomer **1** prior to polymerisation; copolymer **3** (Scheme 1) was prepared in thf using the experimentally-straightforward McCullough Grignard metathesis method,[6,7] and was purified by precipitation into MeOH, followed by sequential Soxhlet extraction [see ESI (i)].† A 69% yield of $CHCl_3$ soluble fraction was obtained, which had >97% head-to-tail regioregularity, measured by comparing the major thienyl–CH_2 triplet resonance at δ = 2.82 p.p.m. with the minor multiplet (due to non-head-to-tail thienyl–CH_2 moieties) centred at 2.54 p.p.m.[8] Integration of the –CH_2OH resonance and comparison with the thienyl–CH_2 resonance gave a monomer ratio in the polymer of 1:8.5 **1**:**2**; it is possible that polymer chains richer in **1** were more soluble in the other solvents used in the Soxhlet procedure. Biotin was grafted to copolymer **3** using dicyclohexylcarbodiimide (DCC)-mediated, 4-(dimethylamino)pyridine (DMAP)-catalysed coupling in $CHCl_3$ [see ESI (ii)].† The biotin-functionalised copolymer, **4**, was purified by washing with water, followed by Soxhlet extraction with MeOH overnight, to remove excess reagents and dicyclohexylurea. Integration of the –CH_2OC(O)– (δ = 4.10 ppm) and residual –CH_2OH resonances in the ^{1}H NMR spectrum of **4** indicated



Scheme 1

† Electronic supplementary information (ESI) available: syntheses and characterization of polymers **3** and **4**. See http://www.rsc.org/suppdata/cc/b4/b408935a/

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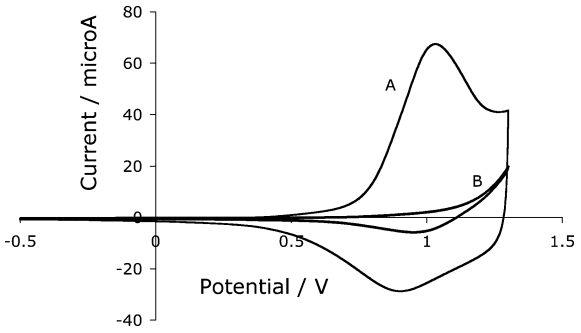

Fig. 1 Cyclic voltammograms of a drop-cast film of polymer **4** on a 0.13 cm^2 Pt disc electrode, cycled at 10 mV s^{-1} in 0.1 M Et_4NBF_4/CH_3CN, (A) prior to exposure to avidin and (B) after incubation in 1 cm^3 of a 0.1 M NaCl/10 mM edta buffer solution containing 10^{-9} mol avidin. The polymer-modified electrode was washed gently with fresh buffer, dried in a stream of N_2, washed with CH_3CN and transferred back to the CH_3CN electrolyte prior to cycling.

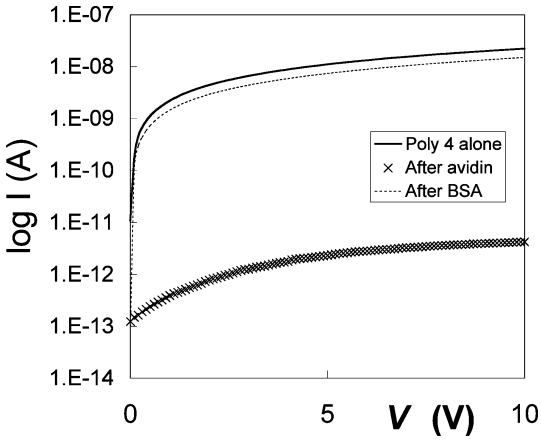

Fig. 2 *IV*-characteristics of a spin-coated film of polymer **4** prior to buffer exposure (full line), after exposure to 10^{-8} mol of BSA in 1 cm^3 of 0.1 M aq. NaCl/10 mM edta buffer (broken line), and after exposure to 10^{-9} mol of avidin in 1 cm^3 of buffer (crosses).

that *ca.* 40% of the –OH groups had been esterified by biotin [see ESI (iii)].†

Both **3** and **4**, dissolved in 1:1 $CHCl_3$:dmso (1 mg in 2 cm^3, equivalent to 2.7×10^{-7} mol. biotin in the case of **4**), gave purple solutions with λ_{max} for the π–π^* absorption at 515 nm, and shoulders at 555 and 600 nm. This is characteristic of these regioregular polymers in 'poor' solvents, in which a degree of polymer self-assembly (and consequent π–π stacking) takes place;[9] the solvent system was chosen for compatibility with both the polymer, and avidin in aqueous buffer. On addition of 1.5×10^{-9} mol of avidin (dissolved in 0.1 cm^3 0.1 M NaCl/10 mM edta aqueous buffer) to the polymers, virtually no change in λ_{max} was observed for polymer **3**, but a gradual change (over 15 min) to a yellow colour (λ_{max} for the π–π^* absorption at 472 nm; no vibronic splitting) occurred for **4**. Furthermore, addition of bovine serum albumin (BSA; 10^{-8} mol in 0.1 cm^3 buffer) to the solution of **4** also caused almost no change [see ESI (Figure)].† This is consistent with specific binding of avidin to the biotin moieties of **4**, causing a dissociation of interpolymer π–π stacking, and significant thiophene–thiophene inter-ring torsion, increasing the energy of the π–π^* transition. This result is comparable with that for the poly(3-alkyl-4-alkoxy)thiophene derivative already described.[17]

We have also shown that polymer **4** can be used to fabricate both electrochemical and solid-state avidin detectors. Fig. 1 shows cyclic voltammograms of a drop-cast film of polymer **4** on a Pt disc electrode in 0.1 M Et_4NBF_4/CH_3CN, before and after exposure to 10^{-9} mol of avidin in 1 cm^3 of aqueous buffer. The large decrease and positive shift in the oxidation wave after exposure to avidin is consistent with the blocking of ion ingress/egress on polymer switching by the protein. No significant change in the electrochemistry of a film of **4** was observed after exposure to 10^{-8} mol of BSA in 1 cm^3 buffer, indicating that the specific biotin–avidin interaction was responsible for the electrochemical change. The minimum amount of avidin sufficient to cause a detectable shift and diminution of the redox wave of **4** was 5×10^{-14} mol, using an electrode of area 0.13 cm^2; experiments using microelectrodes to lower the detection limit are in progress.

A simple solid-state device was fabricated by spin-coating *ca.* 100 nm of polymer **4** onto two gold finger electrodes (0.1 × 1 cm; 0.1 cm apart) deposited onto a hexamethyldisilazene-treated glass substrate. Fig. 2 shows the effect of avidin exposure on the *IV*-characteristics (shown as log *I*; the polymers obeyed Ohm's law). While exposure of the polymer to BSA (10^{-8} mol in 1 cm^3 buffer) causes a very small change in conductivity, exposure of this device to excess avidin (10^{-9} mol in 1 cm^3 buffer) causes a lowering of conductivity by nearly four orders of magnitude.

In summary, this biotin-functionalised regioregular polyalkylthiophene responds to avidin binding with a drastic change in colour in solution, and with marked electrochemical and chemoresistive changes, all three modes being applicable to sensor fabrication.

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Notes and references

1 D. T. McQuade, A. H. Hegedus and T. M. Swager, *J. Am. Chem. Soc.*, 2000, **122**, 12389.
2 S. J. Higgins, *Chem. Soc. Rev.*, 1997, **26**, 247.
3 M. Leclerc, *Adv. Mater.*, 1999, **11**, 1491.
4 J. Roncali, *Chem. Revs.*, 1992, **92**, 711.
5 J. Roncali, *J. Mater. Chem.*, 1999, **9**, 1875.
6 R. S. Loewe, S. M. Khersonsky and R. D. McCullough, *Adv. Mater.*, 1999, **11**, 250.
7 R. S. Loewe, P. C. Ewbank, J. S. Liu, L. Zhai and R. D. McCullough, *Macromolecules*, 2001, **34**, 4324.
8 R. D. McCullough, *Adv. Mater.*, 1998, **10**, 93.
9 T. Yamamoto, D. Komarudin, M. Arai, B. L. Lee, H. Suganuma, N. Asakawa, Y. Inoue, K. Kubota, S. Sasaki, T. Fukuda and H. Matsuda, *J. Am. Chem. Soc.*, 1998, **120**, 2047.
10 H. Sirringhaus, P. J. Brown, R. H. Friend, M. M. Nielsen, K. Bechgaard, B. M. W. LangeveldVoss, A. J. H. Spiering, R. A. J. Janssen, E. W. Meijer, P. Herwig and D. M. deLeeuw, *Nature*, 1999, **401**, 685.
11 N. M. Sedghi, G. R. Lloyd, M. Raja, W. Eccleston, R. Di Lucrezia and S. J. Higgins, *J. Appl. Phys.*, 2002, **92**, 1441.
12 L. Zhai, R. L. Pilston, K. L. Zaiger, K. K. Stokes and R. D. McCullough, *Macromolecules*, 2003, **36**, 61.
13 N. Reitzel, D. R. Greve, K. Kjaer, P. B. Hows, M. Jayaraman, S. Savoy, R. D. McCullough, J. T. McDevitt and T. Bjornholm, *J. Am. Chem. Soc.*, 2000, **122**, 5788.
14 M. Wilchek and E. A. Bayer, *Anal. Biochem.*, 1988, **171**, 1.
15 M. Wilchek and E. A. Bayer, in *Avidin-Biotin Technology*, ed. J. N. Abelson and M. I. Simon, San Diego, CA, 1990.
16 M. D. Savage, G. Mattson, S. Desai, G. W. Nielander, S. Morgensen and E. J. Conklin, *Avidin-Biotin Chemistry: A Handbook*, Pierce Chemical Co., 1992.
17 S. Bernier, S. Garreau, M. Béra-Abérem, C. Gravel and M. Leclerc, *J. Am. Chem. Soc.*, 2002, **124**, 12463.
18 M. Lanzi, P. Costa-Bizzarri, C. Della-Casa, L. Paganin and A. Fraleoni, *Polymer*, 2003, **44**, 535.