Electrochemically controlled micropatterning of immobilised species on functionalised electrode interfaces

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The reactivity of an activated ester group in a suitably functionalised electrodesposited conducting polymer can be modulated electrochemically, providing a mechanism for the patterning of immobilised species at discrete electrodes.

Conducting polymer matrices have previously been used extensively for the immobilisation of active biological species including enzymes and DNA fragments,[1] as well as other ligand binding species, including *e.g.* crown ethers.[2] One advantage of the technique concerns the ability to electrodeposit the polymer matrix in specific, electronically addressable arrays, thereby providing the possibility of selective immobilisation of different species at different places within an electrode array (*i.e.* to pattern a bound species). Under these circumstances the polymer matrix must either be deposited sequentially,[3] with intervening coupling reactions, or sequentially activated,[4] for example by light passing through a photolithographic mask.

When considering the various methods that can be used for biomolecule immobilisation at a biosensor interface, reaction between a polymer-bound activated ester and a nucleophilic amine (*e.g.* a biomolecule) has a number of advantages, including minimisation of side reactions and high reaction yields. We now describe a method of promoting or inhibiting the immobilisation of such a species onto a suitably functionalised conducting polymer matrix, involving the (simple) use of an applied electrochemical potential to modulate the reactivity of an activated ester group. We demonstrate this by using electrochemistry, XPS and FTIR spectroscopy to characterise the immobilisation of two model species, hexylamine and 2-ferrocenyl ethylamine ($FcCH_2CH_2NH_2$). These complementary surface-sensitive techniques allow us to investigate both the interfacial and bulk properties of the polymer.

The activated polymer investigated in this study is derived from the pentafluorophenyl ester of 3-pyrrolylpropionic acid (PFP). Thin films of this polymer are prepared by electropolymerisation of the monomer at potentials in excess of 1.0 V (*vs.* Ag/AgCl) in MeCN.[5] Electrochemical doping and undoping of the polymer occurs at 0.75 V, a potential which is significantly higher than the 0.65 V required to dope the unactivated parent polymeric species, poly(3-pyrrolylpropionic acid). This shift in redox potential suggests that the energy levels in the conjugated polymer backbone are perturbed either by electronic distributions in the carbonyl region of the N-pendant group, or more probably by steric effects concomitant with the bulkier side group.[6] Here, we explore the possibility that changes *induced* in the distribution of electrons in the polymer backbone, brought about by electrochemical oxidative doping and undoping, could influence the reactivity of the pendant group activated ester.

Doped and undoped films of poly(PFP) were immersed in DMSO solutions containing a nucleophilic amine species and the results of the experiments, described below, indicate that the rates of both aminolysis and hydrolysis are significantly reduced when oxidatively doped poly(PFP) is exposed to nucleophile-containing solutions. To assist in evaluating the extent of aminolysis of poly(PFP), 2-ferrocenylethylamine, a redox centre containing moiety, was used as a model nucleophile, due to its characteristic electrochemical and Fe(2p) XPS spectroscopic signature. The redox peaks at *ca.* 0.42 V in the cyclic voltammograms of Fig. 1(*a*), due to the Fc/Fc^+ couple, illustrate the degree of coupling which occurs upon holding a poly(PFP) electrode in a 1 mM 2-ferrocenylethylamine solution at 0 V. When the poly(PFP) electrode is held in the 2-ferrocenylethylamine solution at 0.77 V, there is a complete absence of these redox peaks, [Fig. 1(*b*)]. Fig. 1(*c*) shows the voltammogram arising from an electrode previously held in the doped state in the $FcCH_2CH_2NH_2$ solution, after reimmersion, following undoping, in the nucleophile containing solution. These results clearly show the immobilisation of ferrocene species into the polymer matrix is inhibited by maintaining the polymer in a doped state, and furthermore, that the reactivity is restored following undoping of the polymer.

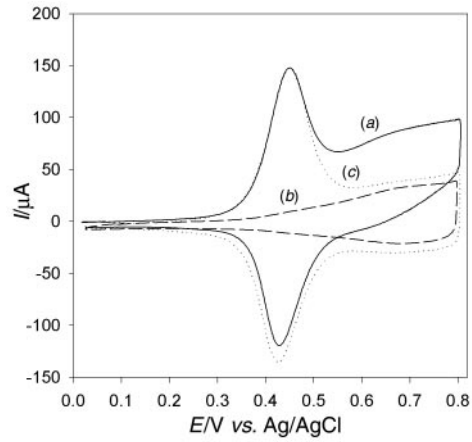

Fig. 1 Cyclic voltammagrams of poly(PFP) films in MeCN after immersion in DMSO solutions containing 1 mM $FcCH_2CH_2NH_2$ for 3 min under various conditions of electrochemical control: (*a*) electrode held at 0 V; (*b*) electrode held at 0.77 V; (*c*) electrode initially held at 0.77 V in $FcCH_2CH_2NH_2$/DMSO solution for 3 min, and then held at 0 V in same solution for 3 min. Electrode areas 1 cm^2; scan rate 20 mV s^{-1}; supporting electrolyte 0.1 M tetraethylammonium perchlorate, for both MeCN and DMSO solutions.

The RAIRS spectra of Fig. 2(*a*)–(*c*) are of a poly(PFP) film [Fig. 2(*a*)], together with poly(PFP) electrodes which have been held in $FcCH_2CH_2NH_2$ solutions at either 0 [Fig. 2(*b*)] or 0.77 V [Fig. 2(*c*)]. These confirm that for the polymer held at 0 V, the ferrocene species becomes covalently attached *via* an amide linkage (loss of the fluorophenyl ester peak at 1790 cm^{-1}, and the subsequent appearance of amide I and amide II peaks at 1650 and 1570 cm^{-1}). The small change from the as-deposited spectrum, for the doped polymer which has been immersed in the nucleophile containing solution at 0.77 V [Fig. 2(*b*)], indicates the activated ester functionality is not lost following maintenance of polymer in the oxidised state. This observation is consistent with the cyclic voltammetry of Fig. 1(*c*) corre-

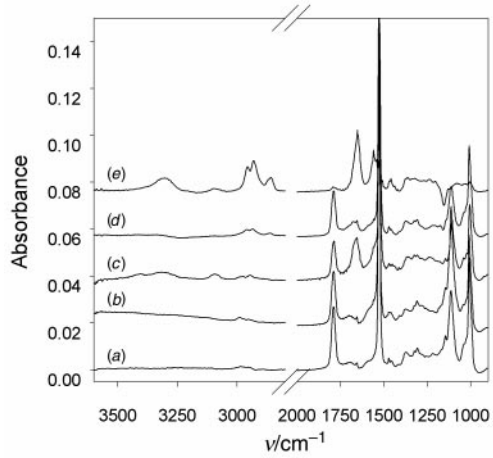

Fig. 2 RAIRS spectra for poly(PFP) films polymerised on evaporated gold electrodes, after immersion in amine containing solutions whilst holding at specified electrochemical potentials (spectra offset for clarity): (*a*) as prepared poly(PFP) film; (*b*) oxidatively doped film immersed in 1 mM $FcCH_2CH_2NH_2$/DMSO for 3 min; (*c*) undoped film immersed in 1 mM $FcCH_2CH_2NH_2$/DMSO for 3 min; (*d*) oxidatively doped film immersed in 0.5 mM hexylamine/DMSO for 3 min; (*e*) undoped film immersed in 0.5 mM hexylamine/DMSO for 3 min.

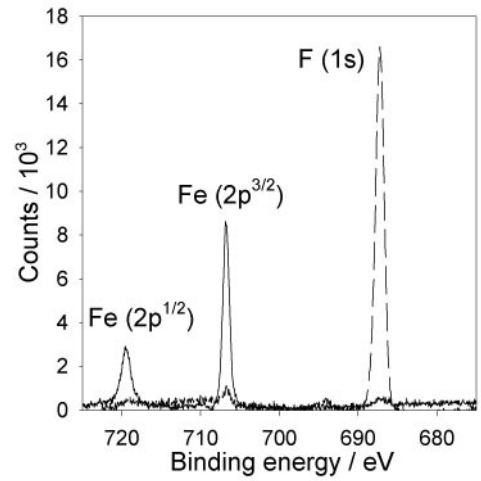

Fig. 3 F(1s) and Fe(2p) XPS spectra of poly(PFP) films after immersion in DMSO solutions containing 1 mM $FcCH_2CH_2NH_2$ and 0.1 M tetraethylammonium perchlorate for 5 min; (---) electrode held at 0.77 V; (—) electrode held at 0 V.

sponding to immobilisation of $FcCH_2CH_2NH_2$ into a previously 'inhibited' polymer film. The short reaction times used for the samples illustrated in Fig. 2 show that partial substitution is possible, if desired; however complete substitution is readily achieved simply by increasing the immersion time. Notably, coupling inhibition in the doped polymer can be maintained for >30 min.

The Fe(2p) and F(1s) XPS spectra of Fig. 3 corroborate the results of Figs. 1 and 2, and show that the amount of ferrocene in the surface layers of the film following immersion of the modified electrode at 0.77 V in the ferrocene ethylamine solution is *ca.* 5% of that found in a fully reacted film, giving a patterning selectivity of 20:1 in this unoptimised system. This low level could not directly be inferred from the lack of signal in the corresponding cyclic voltammogram (Fig. 1) since physisorbed, or partitioned, $FcCH_2CH_2NH_2$ may have exhibited poor electronic communication with the poly(PFP) matrix.

Since $FcCH_2CH_2NH_2$ is in the positively charged, oxidised state at 0.77 V, to investigate whether this coupling inhibition was a consequence of permselectivity effects in the oxidised, cationic membrane, similar potential-dependent coupling experiments were performed with the strong nucleophile, hexylamine. Hexylamine is unoxidised at these potentials (and also uncharged) and thus its permeation into the polymer is not restricted by similar permselectivity effects as may operate for $FcCH_2CH_2NH_2$. As expected, the redox voltammograms of the hexylamine-substituted poly(PFP) show the doping/undoping process now occurs at a lower potential (*ca.* 0.6 V). Figs. 2(*c*) and (*d*) show the RAIRS spectra for poly(PFP) electrodes held in either the undoped or oxidatively doped state respectively whilst immersed in hexylamine–DMSO solutions. These spectra indicate a complete reaction occurs with the undoped poly(PFP) (appearance of amide bands and C–H stretch at 2850 cm^{-1} arising from the alkyl chain) and a limited reaction for polymer films poised in the doped state. It was also noted that coupling inhibition persisted after removal of the applied potential following doping of the polymer at 0.77 V.

Furthermore, it has been found that hydrolysis in basic semi-aqueous MeCN, DMF or DMSO solutions can also be inhibited for *ca.* 30 min by electrode polarisation at 0.77 V. This extended duration compares to less than 5 min for complete hydrolysis when the electrode is held at 0 V, and, as a consequence, this technique may suitable for the nucleophilic coupling of biological species from semi-aqueous organic solvents. Furthermore, using an electrochemical assay to determine the degree of aminolysis by either $FcCH_2CH_2NH_2$ or hexylamine it has been possible to demonstrate selective patterning of $FcCH_2CH_2NH_2$ on a photolithographically fabricated microelectrode, followed by coupling of hexylamine to an adjacent (10 μm separation) polymer modified electrode (at which $FcCH_2CH_2NH_2$ coupling had previously been inhibited).

A functionalised, conjugated polymer film is physically a complex environment and the results to date do not establish a mechanism for the inhibition of these aminolysis and hydrolysis reactions, other than its independence of significant charge permselectivity control. Further mechanistic possiblities which are being investigated include: (i) changes in the bonds strengths in the activated ester group induced by electronic band changes in the conducting polymer backbone associated with doping and undoping; (ii) hindrance of nucleophile attack at the activated ester group induced by molecular conformational changes associated with doping and undoping the polymer backbone; and (iii) changes in the membrane's internal ionic strength associated with doping inhibiting the ingress of hydrophobic nucleophiles.

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