A Bio-electronic Interface using Functionalised Conducting Poly(pyrroles)

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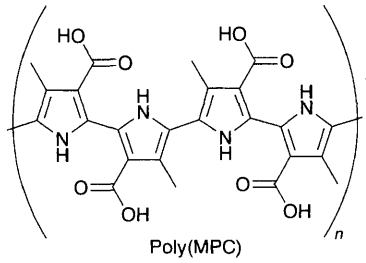
We describe for the first time direct electronic communication between a functionalised, conducting poly(pyrrole) and a redox protein (cytochrome c) in solution, demonstrating that facile, heterogeneous electron transfer is a function both of polymer substituent pattern and backbone conductivity.

The study of the interaction of biological molecules, including proteins and enzymes, with electronic materials has great potential in the field of molecular electronics, with applications in the design of submicron sized electronic components[1] and in the development of novel biosensors.[2] A prerequisite for the design of such a bio-electronic interface is that the materials used should possess high conductivity and good compatibility with the outer shell of the biological species, such that surface interactions do not induce phase separation (as often occurs, *e.g.* in mixtures of polymers). Other adverse phenomena, such as strong (or irreversible) physical adsorption, or protein denaturing effects, which are often observed at conventional conducting electrode substrates, *e.g.* gold or silicon, should also be avoided. Additionally, if such an interface is to have potential application in the design of biosensors, the incorporation of functional groups conferring molecular recognition properties is important.

One approach towards this has been to modify electrodes with functionalised thiols. Seminal work in this area by Hill *et al.* utilises gold electrodes coated with a monolayer of *N*-acetylcysteine or 4,4′-bipyridine in order to achieve fast, reversible and highly selective heterogeneous electron-transfer to cytochrome c in aqueous solution, without electrode fouling.[3,4] However, such monolayers are neither versatile nor physically robust. In our work we have been developing surface modifiers generic to a range of electrode surfaces which have the potential to provide a wide variety of chemical functionalities, whilst being both stable and compatible with thin-film fabrication techniques, such as photolithography.

In this context we have investigated electron-transfer reactions between a well characterised redox protein and an electrode surface modified with a functionalised conducting poly(pyrrole). Here we describe the first example, to our knowledge, of rapid electronic communication between cytochrome c and an electrode coated with an electrochemically grown poly(pyrrole), poly(MPC) (MPC = 3-methyl-4-pyrrolecarboxylic acid).

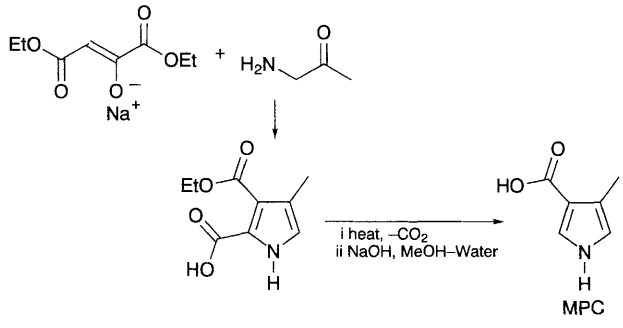
MPC was prepared using methods adapted from the literature,[5,6] according to Scheme 1. Electrochemical polymerisation from acetonitrile solution (20 mmol dm^{-3} MPC, 50 mmol dm^{-3} $[NBu_4][ClO_4]$) under aerobic conditions was facile on Pt, Au or vitreous carbon electrodes. Thin poly(MPC) films (*e.g.* growth charge of 1.5 mC cm^{-2}, corresponding to an approximate thickness of *ca.* 0.3 μm) were grown using a single voltammetric scan (v = 30 mV s^{-1}) up to the base of the pyrrole oxidation wave. Thicker films (> 1 μm) were obtained by maintaining the electrode potential at the minimum value required for current growth (*ca.* +1.0 V *vs.* AgCl/Ag). The 'thick' poly(MPC) films were dark brown (in the reduced form) and were characterised by CV, chronocoulometry and reflectance FTIR spectroscopy [*e.g.* ν(C=O) 1640 cm^{-1}]. The properties of these films were consistent with earlier findings,[7,8] in so far as they have been reported.



Poly(MPC)

An electrode (Pt or Au 2 mm disk) coated with poly(MPC) was washed sequentially with fresh MeCN, distilled H_2O and placed in an aqueous solution of horse heart cytochrome c (10 mg ml^{-1}, 100 mmol dm^{-3} sodium phosphate buffer pH 7.4, 50 mmol dm^{-3} KCl). CV of the cytochrome c solution showed a well defined reversible one electron response attributable to the Fe^{III}/Fe^{II} redox couple of the haem group of the protein, Fig. 1.

The redox potential, $E_{\frac{1}{2}}$ = +52 mV *vs.* AgCl/Ag, compares well with that of the protein observed at a gold electrode modified with a monolayer of *N*-acetyl cysteine (+60 mV)[3] whilst the peak separation, ΔE_p = 80 mV (at 20 °C), remained



Scheme 1

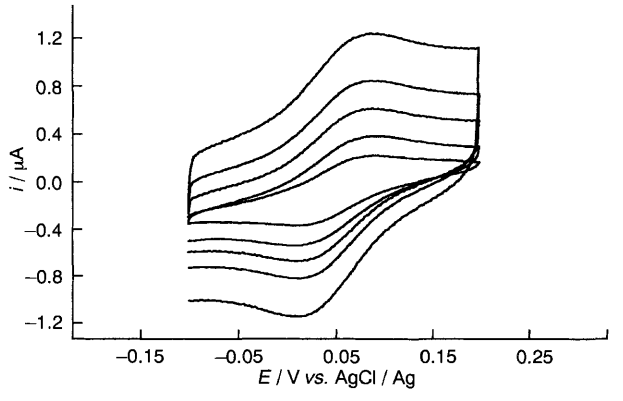


Fig. 1 CV of horse heart cytochrome c (10 mg ml^{-1}) in aqueous phosphate buffer (100 mmol dm^{-3} phosphate, 50 mmol dm^{-3} KCl), pH 7.4, at a 2 mm Au disk coated with a thin film of poly(MPC). Potential scan rates are v = 5, 10, 15, 20 and 30 mV s^{-1} ($E_{\frac{1}{2}}$ = +52 mV, ΔE_p = 80 mV at 20 °C). The polymer film was grown in MeCN solution (20 mol dm^{-3} MPC, 50 mmol dm^{-3} $[NBu_4][ClO_4]$) with a single voltammetric scan from 0.0 up to +1.1 V *vs.* AgCl/Ag (v = 30 mV s^{-1}), the growth charge was 1.5 mC cm^{-2} which corresponds to an approximate film thickness of *ca.* 0.3 μm.

independent of potential scan rate below ν = 30 mV s^{-1}. The voltammetric anodic peak current (ip_a/μA) showed a linear dependence on the square root of the potential scan rate [$\nu^{\frac{1}{2}}$(V $s^{-1})^{\frac{1}{2}}$], Fig. 2, demonstrating that the electron-transfer reaction is diffusion controlled rather than that of an adsorbed species. This is further supported by the observation that peak currents at the bio-polymer assembly are similar in magnitude to those at functionalised monolayers. At scan rates ν > 30 mV s^{-1}, ip_a is smaller than expected suggesting that the current is limited by adsorption/desorption rates of the protein at the polymer. The modified electrode was incubated overnight (at 4 °C) in the protein solution but no irreversibly adsorbed protein was detected by subsequent voltammetry in fresh, protein free, buffer solution.

In order to determine that facile electron transfer is both a function of polymer substituent and electronic conductivity, a number of control experiments were undertaken involving other functionalised pyrroles. A modified electrode prepared under similar conditions was coated with a polymer of the ethyl ester of MPC, (MPC-Et). The poly(MPC-Et) coated electrodes were also fully characterised [*e.g.* ν(C=O) 1682 cm^{-1}) but showed no voltammetric response when immersed in cytochrome c solution. This is consistent with expectation since this polymer does not possess the carboxylic acid groups required to orient the protein and enable electron transfer.[3] Similarly no voltammetric response was observed either at an electrode coated with unsubstituted poly(pyrrole), or at the freshly polished (0.3 μm alumina paste), uncoated electrode substrate.

To exclude the possibility that electron transfer takes place at the substrate surface exposed by 'pin-hole' defects in the polymer film, the poly(MPC) coated electrode was incubated for 10 min in an aqueous solution of H_2O_2 (30%). The modified electrode was then removed, thoroughly washed with distilled H_2O and returned to the protein solution. No subsequent voltammetric response for the cytochrome c was observed. Since it is known that H_2O_2 causes the irreversible destruction of electronic conductivity in poly(pyrroles),[9] this result implicates the polymer backbone in the charge-transport mechanism and excludes the possibility that exposed substrate, *i.e.* polymer 'pin-holes', acts as reaction sites. A similar result was also obtained after electrochemical over-oxidation of the polymer in acetonitrile solution. That the H_2O_2 solution does not dissolve the polymer layer was verified in a separate experiment by specular reflectance FTIR spectroscopy. The FTIR spectrum of a planar Au electrode (Au coated glass slide) coated with poly(MPC) was recorded before and after soaking in peroxide solution. The spectra were identical.

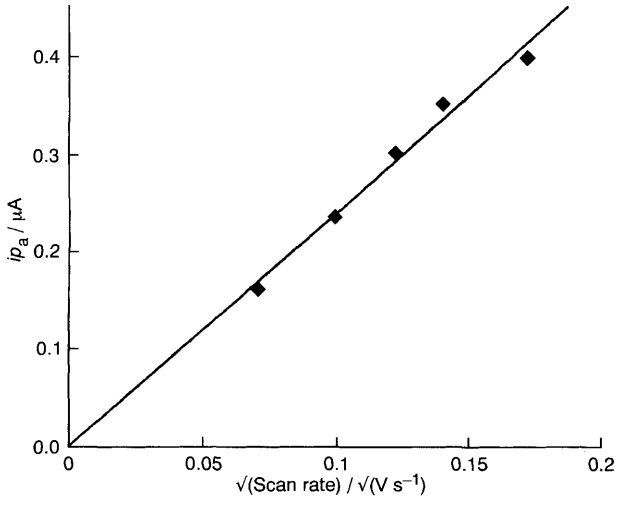


Fig. 2 Plot of voltammetric peak current ip_a (measured from Fig. 1) *vs.* square root of potential scan rate. Linear response at slower scan rates demonstrates diffusion controlled character.

The interfacial charging current of the bio-polymer electrode assemblies shows an increase with increasing film thickness (measured by chronocoulometry), whilst the magnitude of the voltammetric peak current (ip_a) remained relatively unchanged. This suggests that the protein does not penetrate the film but that the heterogeneous electron-transfer process takes place at the polymer solution interface. Interestingly, this is in contrast to recently reported work by Garnier *et al.*[10] who described molecular recognition between a poly(pyrrole), functionalised with a peptide motif, and a carboxypeptidase enzyme. Here the interaction takes place over the bulk of the polymer and implies an open polymer structure through which the enzyme is free to diffuse.

Exclusion of cytochrome c from the partially doped poly(MPC) film may result from electrostatic repulsion due to generation of positive charge on the polymer backbone during doping, or may be because of restricted diffusion of the protein within the polymer. Varying polymerisation conditions in order to alter polymer morphology, however, produced no measurable effect.†

It is clear that polymer functionality as well as backbone conductivity are crucial factors in this interaction. In the only other description of a similar bio-polymer interaction, between poly(5-carboxyindole) and cytochrome c,[11] the polymer film is susceptible to dissolution at pH > 5.5, whereas the system that we describe shows no such tendency and the modified electrodes are chemically stable and physically robust. In addition, the nature of substitution chemistry at the pyrrole monomer offers a much wider variety of functional possibilities including electron-transfer polymers derived from functionalised oligomers.

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Footnote

† For example, variations in growth rate conditions, different solvents, including propylene carbonate and trifluoroethanol, and larger counterions, *e.g.* [*p*-$Me(C_6H_4)SO_3$]$^-$.

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