Electrochemical characterization of monolayers of a biotinylated polythiophene: towards the development of polymeric biosensors

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Ultrathin films of an electrostatically-modified biotinylated polythiophene have shown interesting electrochemical properties which lead to the detection of femtomoles of the protein avidin in aqueous electrolytes.

Conjugated polymers (polyanilines, polythiophenes, polypyrroles, poly(phenylene vinylene)s, polyfluorenes, *etc.*) and related oligomers have become an important class of organic materials due to their unusual electrical and optical properties which lend themselves to the development of various devices in the areas of micro-electronics, electro-optics, photonics, sensors, genomics, *etc.*[1–7] In this respect, functionalized polythiophenes have been the subject of many investigations since some of them combine good processability and environmental stability with an ability to detect, transduce, and amplify various physical or chemical information into an electrical or optical signal.[5–7] These novel organic materials may serve in integrated sensory devices where the optical or electrical signal will not require any external tagging procedures but would come directly from the responsive polymeric supports. In order to achieve these goals, simple, specific, highly sensitive, and miniaturized polymeric devices must also be developed.

Along these lines, we have recently reported the easy preparation of electroactive thin films of functionalized polythiophenes through electrostatic interactions.[8] The deposition of ultrathin films (even monolayers) could be very important for solid-state biosensors since it is believed that the interaction between large biochemical molecules and the binding sites present within the electroactive materials will mainly occur at the interface. In the present study, we have electrochemically characterized the stability and the sensitivity of such simple polymeric assemblies for the future development of microarrays of polymeric sensors.

Following previously reported procedures,[8] ultrathin films of 50% pre-neutralized poly(2-(4-methyl-3-thienyloxy)ethanesulfonic acid) (PMTOES) have been easily transfered onto aminosilane-treated ITO electrodes.† Depending upon the nature of the side chains, various functionalized polythiophenes can be obtained but in the course of the present study, only biotinylated polythiophenes will be analyzed (see Fig. 1). As shown in Fig. 2, these electroactive ultrathin films have been characterized in an aqueous electrolyte (pH = 6–7) and have revealed a clear and reversible redox (related to a doping/undoping electrochemical process) process around 0.4 V *vs.* SCE. This quasi-reversible redox process occurs at a much lower potential than that previously reported in an organic electrolyte.[8] This could be related to the hydrophilic nature of the polymeric salt which allows very efficient wetting of the polymer in aqueous solutions. The good electrochemical activity of this polythiophene derivative in water opens the door to electrochemical detection in real biological systems. Various sizes (1 to 100 mm^2) of electrodes have been analyzed and a good correlation has been obtained between the surface of the electrode and the integrated charge exchanged during the redox process. On average the oxidative charge obtained by integration of the 0.44 V peak is 0.24 μC mm^{-2}. The number of electrons exchanged per repeat unit is difficult to assess but, on the basis of previous studies on electroactive polythiophenes,[9,10] it is reasonable to assume an exchange of one electron per three repeat units (*i.e.* a doping level of 33%). This rough estimate corresponds to a molecular density of 22 Å^2 per repeat unit. This value is in good agreement with recent results (21–23 Å^2) obtained with self-assembled polythiophene monolayers.[11]

The electrochemical responses of these biotinylated electrodes have been investigated upon addition of the protein avidin. This protein is built from four identical subunits (for a total M.W. = 68 000) and has a strong affinity for biotin.[12] This strong affinity between avidin and biotin is related to an exceptionally high binding constant of 10^{15} M^{-1}, which

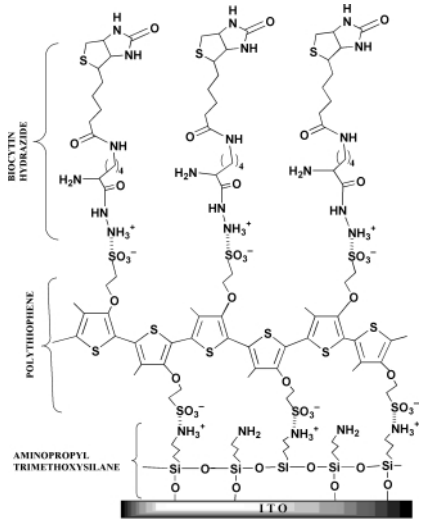

Fig. 1 Schematic and idealistic description of a biotinylated polythiophene electrode.

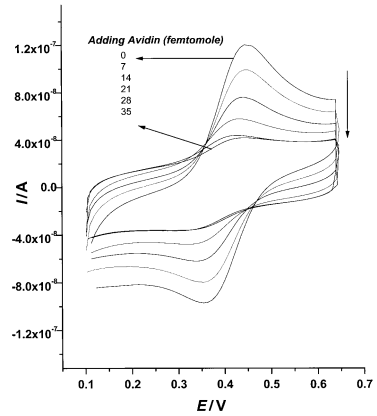

Fig. 2 Cyclic voltammogram of a 1 mm^2 biotinalyted polythiophene electrode in a 0.1 M KCl aqueous solution upon addition of aliquots of avidin, at room temperature. Scan rate of 20 mV s^{-1} *vs.* SCE.

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corresponds to a binding energy of 20 kcal mol^{-1}. For these reasons, the biotin/avidin couple has been extensively used in different types of biosensors[8,12–19] and is utilized here as a model compound for the future design of various types of sensors based on functionalized polythiophenes.

Interestingly a 1 mm^2 biotin-functionalized polythiophene electrode shows a clear modification of its cyclic voltammogram‡ (a decrease of the electrochemical activity) upon addition of as low as 7×10^{-15} mole of avidin in the aqueous electrolyte (Fig. 2 and 3). Saturation is almost obtained at 3.5×10^{-14} mole of avidin. Modified electrodes of area 1 cm^2 have shown similar electrochemical effects upon addition of aliquots of avidin but at concentrations multiplied by a factor of 100 (which corresponds to their surface ratio). A similar decrease of the electrochemical activity has been observed in nucleobase-functionalized polythiophenes[20] and could be explained by a decrease of the interfacial electron-transfer rates between the polymer and the electrode, due to the binding of the protein.[21] In other words, the bound avidin may shield the electroactive polymer and therefore affect the electronic exchanges between the electrode, the polymer and the electrolyte. First experiments on similar biotinylated electrodes in acetonitrile have revealed an increase of the redox potential[8] which had been explained by a twisting of the backbone upon complexation, but this mechanism does not seem to be dominant in aqueous electrolytes. Another explanation could be a release of the polymer from the electrode upon avidin binding. A similar mechanism was recently proposed for fluorescent conjugated polyelectrolytes.[22] It is clear that covalently-attached polythiophene derivatives would shed some light on the mechanism involved in the detection and these new polythiophene derivatives are currently being developed in our laboratory. It is also interesting to note that polythiophene-modified electrodes without biotin moieties (50% pre-neutralization of the sulfonic acid polymer with sodium hydroxide) do not show any significant modification of their electrochemical properties. These results also give clear evidence of the electrochemical and mechanical stability of such polymeric electrodes. Indeed, although these polythiophene derivatives are soluble in water, it seems that the multiple electrostatic interactions along the backbone are strong enough to avoid any significant release of the ultrathin polymeric film when immersed in aqueous electrolytes, the electrochemical activity being reproducible even after hours of immersion in the electrolyte.

From Fig. 3, it is also possible to assume that about 24% of the thiophene units are affected by the presence of 7×10^{-15} mole of avidin and that could mean that one avidin molecule affects about 250 repeat units. This amplification factor is similar to that calculated from biochromic measurements.[8] It is believed that the present amplification factor is more or less related to the difference in size between the protein avidin and one repeat thiophene unit. Indeed, as mentioned above, it is possible to determine that one thiophene unit occupies a surface of 22 $Å^2$, and from this amplification factor of 250 that could indicate that avidin occupies a surface of roughly 55 nm^2. These dimensions are in the same range as those determined by X-ray analyses for a parent protein (streptavidin, M.W. = 60 000 with dimensions of $4.5 \times 4.5 \times 5.8$ nm).[13]

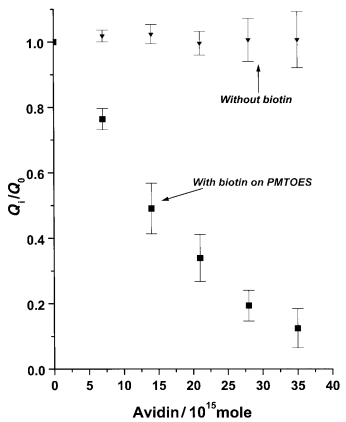

Fig. 3 Relative electroactivity (integrated charge exchanged during the oxidative 0.44 V peak) upon addition of avidin for a monolayer made of a 0.5:1 complex between sodium hydroxide and poly(2-(4-methyl-3-thienyloxy)ethanesulfonic acid) (▼) and a monolayer made of a 0.5:1 complex between biocytin hydrazide and poly(2-(4-methyl-3-thienyloxy)ethanesulfonic acid) (■).

In conclusion, this simple methodology, based on non-covalent interactions between water-soluble amine-bearing molecules and partially neutralized poly(2-(4-methyl-3-thienyloxy)ethanesulfonic acid), has allowed the efficient preparation of stable, highly sensitive, functionalized polythiophene electrodes and constitutes a promising platform for the future development of novel electrochemical biosensor arrays.

Notes and references

† Poly(2-(4-methyl-3-thienyloxy)ethanesulfonic acid) (PMTOES) was prepared following procedures described in previous publications.[8,23] 3-Aminopropyltrimethoxysilane (Aldrich), biocytin hydrazide and avidin (Pierce) were used as received. Amino-functionalized silanized ITO electrodes were prepared following well established procedures.[8,24,25] The electrostatic transfer of 50% pre-neutralized (performed with biocytin hydrazide or sodium hydroxide) poly(2-(4-methyl-3-thienyloxy)ethanesulfonic acid) was carried out by dipping the glass slides into 0.01–0.001 M aqueous solutions of the resulting polymer.

‡ Cyclic voltammograms were obtained with a Solartron 1287 potentiostat/galvanostat driven by Corrview software. The integrated charges during redox processes have been digitally calculated using this software. Satured calomel electrode (SCE) and a platinum plate were used as reference and auxiliary electrodes, respectively. Electrochemical measurements were performed at 20 mV s^{-1} in a 0.1 M KCl aqueous solution. All cyclic voltammograms shown in the present figures are the third ones in a series of three electrochemical cycles. Addition of aliquots of aqueous solutions of avidin were carried out directly in the electrolyte near the biotinylated electrodes. All reported electrochemical data are the averaged responses of five different electrodes.

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