Synthesis of a biotin functionalized pyrrole and its electropolymerization: toward a versatile avidin biosensor

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The synthesis of a biotinylated pyrrole and its copolymerization with pyrrole for the purpose of constructing an electronic conducting polymer (ECP) avidin sensor is reported, using fluorescence detection to determine the efficiency of the sensing process.

Electronic conducting polymers (ECP) have many interesting features that make them ideal candidates for sensing devices, and have thus became the subject of many investigations. In addition to their ease of electrodeposition, these polymers can be functionalized by insertion of specific moieties[1] that provide desirable features such as the selective recognition of alkaline cations.[2] The application of ECP in the field of sensors has also been extended to biological species,[3–5] including DNA, peptides and enzymes. The detection of these species is based on the specific recognition between a biological moiety that is inserted in the ECP film, and its complementary target in solution. The subsequent recognition is detected either by radioactive labels,[5] or by a significant change in the electrochemical[3,4] or spectrochemical response[6] of the ECP.

An important aspect of these biosensors is the immobilization of desired biological species in the ECP film. This has been accomplished by the entrapment of the species in the polymer matrix during electrodeposition, and by the use of grafted monomers which are then copolymerized with ungrafted monomers. In the case of the former, the electropolymerization conditions must be compatible with the stability of the biomolecule; in addition for the latter case, specific conditions must be developed for each of the grafted species. These approaches were successfully carried out for the construction of DNA biochips.[7]

A recent approach for immobilization is postpolymerization functionalization. In this case, a two-step chemical procedure is needed. First, starting monomers with an activated ester are grafted onto the polymer that will form the ECP film. Secondly, after electrodeposition the biological species is covalently coupled to the hanging ester *via* a reactive amino group.[8]

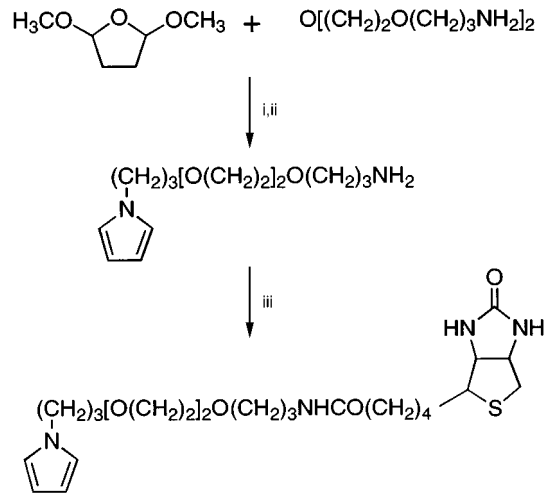
Here we report an original approach for the insertion of biochemical entities in the ECP film *via* the synthesis of a biotinylated polypyrrole. Because of the biotin–avidin affinity, biochemical entities bearing avidin units can bind to the biotin units grafted on the polypyrrole network. This approach allows immobilization of biomolecules on the ECP surface in an easy one-step process, without the use of harsh chemical reagents, since the biotin–avidin system requires only mild immobilization conditions.

The biotinylated polypyrrole film described here is a copolymer electrosynthesized from pyrrole and biotinylated pyrrole, in which the biotin is linked to the nitrogen atom of a pyrrole unit by a hydrophilic spacer arm. The choice of this spacer arm is crucial. It has to be long enough to ensure unrestricted recognition of the biotin by avidin, and a length of eleven atoms or more is deemed necessary.[9] In addition, its chemical composition is important in order to guarantee solubility during the preparation of the active layer, and to achieve good solvation of biotin in order to optimize the recognition. We thus selected a spacer with a hydrophobic/hydrophilic balance originating from three oxygen atoms and ten methylene groups.

The synthesis of the biotinylated pyrrole (Scheme 1) is achieved by coupling an aminoalkylpyrrole and a biotin entity. The aminoalkylpyrrole synthesis was carried out using the reaction pathway described by Jirkowsky and Baudy.[10] While the yields we obtained were consistent with the reported values for the ethanediamine, the equivalents of diamine and the reflux time were increased from 1 to 3 equiv. and 1 to 5 h, respectively. In the second step, the amino group was reacted with the activated ester of biotin to produce the biotinylated pyrrole in 54% yield after purification by column chromatography.

The copolymerization of the film was carried out on microelectrodes (50 × 50 μm) arrayed on a silicon chip, using different ratios of biotinylated pyrrole to pyrrole monomers (from 5×10^{-3} to 5×10^{-7}). The presence and activity of biotin to avidin was verified by incubating the film with streptavidin–phycoerythrin (Aldrich) and measuring the fluorescence using a microscope equipped with a DCC camera. We used a conjugate between streptavidin and a fluorescent phycobiliprotein, namely R-phycoerythrin, in order to maximize fluorescence and to minimize quenching. The support was previously washed by a phosphate buffer then blocked by Denhardt's reagent‡ in order to avoid non-specific adsorption of the avidin; proteins[11,12] are readily adsorbed onto polypyrrole surfaces without this pretreatment.

The results of these measurements on the microelectrode array are summarized in Fig. 1. The intensity of the fluorescence



Scheme 1 *Reagents and conditions*: i, 4,7,10-trioxatridecane-1,13-diamine (3 equiv.), AcOH, dioxane, reflux, 5 h, 32%; ii, 10% KOH, reflux, 5 h, 65%; iii, D-biotin *N*-hydroxysuccinimide ester (1equiv.), DMF, room temp., 16 h, 54%

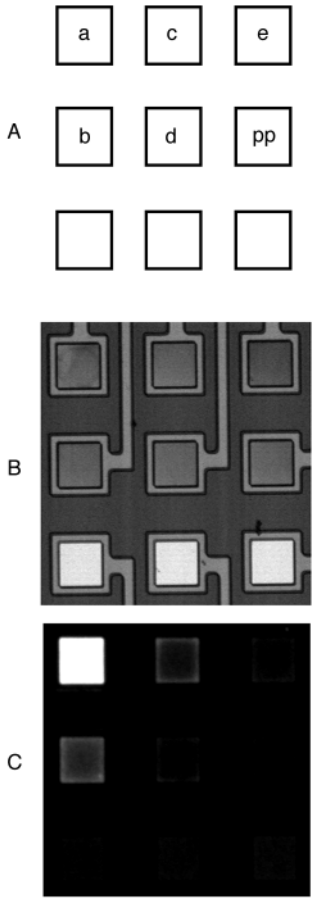


Fig. 1 (A) Pattern of the biotin repartition on the microelectrode array. Electrosyntheses were carried out at 100 mV s^{-1} by repetitive potential scans between −0.35 and 0.85 V *vs.* SCE of 20 mM pyrrole and a decreasing amount of biotin pyrrole with a synthesis charge of 18 mC cm^{-2} (about 60 nm thickness) in 0.1 M $LiClO_4/H_2O$ containing 3% of MeCN on microelectrodes (50 × 50 μm^2) arrayed on a silicon chip. Biotinylated polypyrrole synthesized in presence of (a) 100, (b) 10, (c) 1, (d) 0.1 and (e) 0.01 μM of biotin pyrrole respectively; (pp) = polypyrrole. Each electropolymerization was followed by a thorough washing step and blocking step (see text). (B) Differential interference contrast view of microelectrodes to check the effective deposition of a biotinylated polypyrrole film on the plots. (C) Fluorescence results after a revelation process using a solution of streptavidin–R-phycoerythrin followed by washing in a phosphate buffer.

is related to the amount of biotin involved in the copolymerization reaction and yields the amount of accessible biotin at the surface of the film. The measured fluorescence intensity decreased with a decreasing biotinylated pyrrole to pyrrole ratio. Taking the area marked (a) as the standard, the intensity decreased to 18 and 10.5% of this value when the ratio was decreased to 1:10 and 1:100 [areas (b) and (c)], respectively. In the case of the pure polypyrrole film [without biotin, indicated by region (pp) in Fig. 1(A)], a weak fluorescence was still observed, but only about 2% of the value for (a) and noticeably weaker than the thousand-fold dilution. This indicates that the immobilization of the avidin conjugate on the different dots is specific of the biotin–avidin interaction.

In conclusion, we have reported a simple way of preparing an ECP active layer that is capable of the recognition of avidin conjugates, which would facilitate study of the behavior of many commercial biomolecules. Indeed, this system is very versatile due to the availability of a wide variety of avidin conjugates, which would allow for the elaboration of a large number of biosensors. However, in order to use this sensor on real biological samples, the signal/noise ratio should be increased. In addition, this method has the advantage of miniaturization *via* the use of electrochemical addressing of the electrodeposition.

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Notes and References

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‡ The microelectrodes were dipped for 10 min at room temperature in Denhardt's reagent, composed of 0.02% Ficoll, 0.02% polyvinylpyrrolidone and 0.02% BSA. The dots were then rinsed with a buffer solution and incubated for 5 min in the dark in a solution of 5% streptavidin–R-phycoerythrin. After washing, the fluorescence was recorded for 1 s with a microscope equipped with a CDD camera.

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