Toward Bioelectronics: Specific DNA Recognition Based on an Oligonucleotide-Functionalized Polypyrrole

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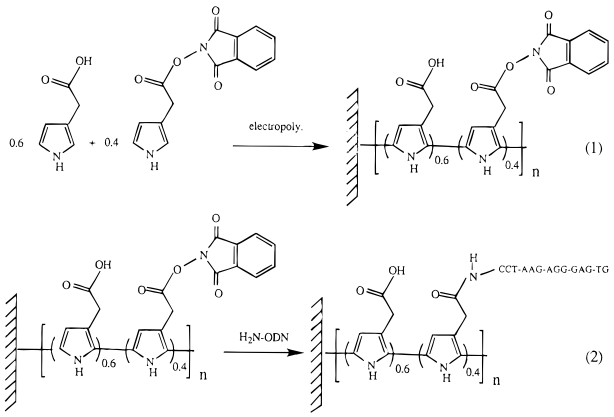
In recent years there has been a growing interest in diagnosing genetic diseases and the detection of human lymphocyte antigen (HLA) groups.[1,2] The diagnostic methods are generally based on hybridization of oligonucleotide probes. A single probe can consist of a matrix of dots coated with various oligonucleotides (ODNs) that simultaneously detect a range of DNA sequences. However, it is difficult to deposit the oligonucleotides precisely on these micro-sized spots and improved methods need to be developed to read the information that is written to the dot matrix. Various techniques have been proposed giving access to an "active" array, such as (i) spotting of modified oligonucleotide microdroplets on an activated support,[3,4] (ii) local support photodeprotection, followed by an oligonucleotide coupling,[5] (iii) an electrochemical copolymerization of pyrrole and oligonucleotide substituted pyrrole,[6] and (iv) immobilization of modified DNA onto gold surfaces.[7]

Most sensors are based on radioactive labeled probes,[8] or more recently, biotin,[9] digoxigenin,[10] and fluorescent dyes[11,12] have been used as non-radioactive labels. The polymerase chain reaction[13] is also used in conjunction with labeled probes to detect very small quantities of DNA or RNA. Some major drawbacks with these techniques are their long detection times and the complex laboratory procedures required. Electrochemically active biosensors have also been described that exhibit faster response times.[14−19] Such devices exploit the intercalation of metal chelates with base pairs of double-stranded DNA. This induces a measurable shift in either the chelate oxidation potential or the cathodic current. However, the observed variations of these electrochemical signals are small and the selectivity is very low.

In view of these problems we have designed a biosensor using an electroactive polypyrrole functionalized with an oligonucleotide probe. Specific hybridization of this grafted ODN with its complementary ODN target in solution induces a significant modification in the electrochemical response of polypyrrole and enables a sensitive electrical reading of the recognition process.

The conducting polypyrrole functionalized with bulky oligonucleotides needs to remain highly electroactive in aqueous media for the electrical reading of an ODN hybridization to take place. The first attempts to electropolymerize an ODN-functionalized pyrrole monomer were unsuccessful, owing to large steric hindrances between monomer units during polymerization. Thus, we have used a recently developed synthetic route toward functionalized conjugated polymers[20] which involves a precursor copolymer, poly[(3-acetic acid pyrrole)/(3-*N*-hydroxyphthalimide pyrrole)] (I), bearing the easy leaving group *N*-hydroxyphthalimide. This precursor polymer (I) allows a further direct chemical substitution of its labile ester group by a prosthetic group bearing a terminal amino function. We showed that aminoferrocene easily displaced the *N*-hydroxyphthalimide moieties in I, leading to a highly electroactive poly(3-acetamidoferrocene pyrrole). Furthermore, the electroactivity of the ferrocene probe was confirmed up to a concentration of about 10^{-6} mol cm^{-2}.[20,27]

Precursor copolymer film I shows a high electroactivity in aqueous media, with a reversible oxidation wave at +0.1 V/SCE and a linear dependence of peak current with scan rate.



In a further step 2, an amino-substituted oligonucleotide was grafted on I by a direct chemical substitution of the leaving group *N*-hydroxyphthalimide, in dimethylformamide (DMF) containing 10% acetate buffer at pH = 6.8, during 3 h. The oligonucleotide $^{5'}$CCT AAG AGG GAG TG$^{3'}$ (IV), bearing a terminal amino group on its 5′-phosphorylated position, was kindly provided by Biomerieux. This substitution led to the final functionalized polypyrrole film poly[(3-acetic acid pyrrole)/(3-ODN acetamido pyrrole)] (V), which was carefully washed in DMF and water, to remove any trace of ungrafted ODN.

The presence of the oligonucleotide (IV) pendent groups on the polypyrrole chains (V) was confirmed by its signature infrared frequencies at 695 and 790 cm^{-1} associated with the phosphodiester bond of ODN[21] and by X-ray fluorescence centered on phosphorous. No quantitative determination of the amount of oligonucleotide substituted on the polypyrrole film was achieved.

The modified electrode V was electrochemically characterized in both H_2O−NaCl (0.5 M) and a biological buffered aqueous medium consisting of a mixture of salmon DNA, sodium

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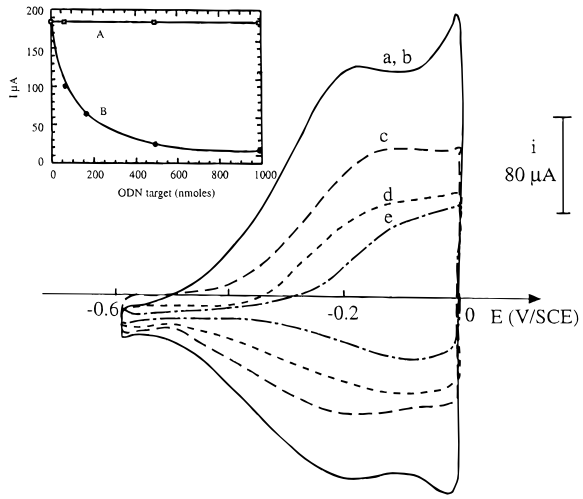

Figure 1. Voltammograms of modified electrode V after incubation in buffered aqueous solutions containing 0.5 M NaCl or PEG (a); in noncomplementary ODN VII (b); and in complementary ODN VI in concentrations of 66 nmol (c), 165 nmol (d), and 500 nmol (e). (inset) Variation of electrode current at constant potential $E = -0.2$ V/SCE as a function of the concentration of noncomplementary ODN VII (A) and complementary ODN VI (B).

phosphate, Tween buffer, and 0.5% of poly(ethylene glycol) (PEG) with average molecular weight of 4000 g at pH = 7. The voltammograms of V in these media are identical (Figure 1a) and exhibit an oxidation peak at −0.2 V/SCE associated with the oxidation (or doping) of the polypyrrole chains. This low oxidation potential value, together with the symmetry of the redox wave, indicates that the ODN-substituted polypyrrole film V is highly electroactive. Furthermore, the absence of any effect due to salmon DNA suggests that the presence of noncomplementary DNA in the electrolytic medium does not affect the electrochemical response of the polymer V.

The modified electrode V was subsequently incubated for 2 h at 37 °C in buffered aqueous solutions of two oligonucleotide targets: (i) an oligonucleotide consisting of 14 bases, bearing the sequence $^{5'}$CAC TCC CTC TTA GG$^{3'}$ (VI) whose structure is complementary to IV, and (ii) a noncomplementary oligonucleotide, consisting of 15 bases, with the sequence $^{5'}$GGT GAT AGA AGT ATC$^{3'}$ (VII). Different amounts of VI and VII varying between 0 and 10^3 nmol were incubated with polymer film V in 5 mL solutions. After incubation, these electrodes were washed using the same buffer and electrochemically characterized in 0.5 M NaCl or buffered aqueous solutions.

When incubated in the presence of the noncomplementary oligonucleotide VII, the electrochemical response of the modified electrode remained unchanged whatever the concentration of VII (see Figure 1b). This indicates that no interaction occurred between the grafted ODN IV and the noncomplementary oligonucleotide VII. Conversely, the complementary ODN VI, which is known to undergo a hybridization reaction under the incubation conditions employed here, leads to a significant change in its voltammetric response (see Figure 1c−e).

These results show that ODN hybridization induces a decrease in the current intensity and a shift to more positive potential of the oxidation wave of V which can be attributed to the bulky conformational modifications made along the conjugated polymer backbone. The aromatic structure of the neutral state allows free rotation of monomer units along the polymer chain, whereas the planar quinoid structure with less degrees of freedom is obtained by polymer oxidation. When the pendent ODNs are hybridized with their complementary partners they become more bulky and stiff. Consequently, the transformation to the quinoid structure of the polymer chain requires more energy, i.e. a higher oxidation potential. Similar effects have previously been observed using conjugated polyheterocycles functionalized with other recognition centers such as cation-complexing crown ethers[22,23] or enzyme-inhibiting peptides.[24]

The response of this bioelectrochemical sensor can be interpreted by analyzing the variation of current at constant potential (−0.2 V/SCE), i.e. in the potential window where ODN hybridization induces the largest modification of the voltammogram. Upon the addition of noncomplementary ODN to the electrolytic medium, no variation of the electrode oxidation current is observed (inset A of Figure 1). In contrast, the addition of the complementary ODN VI decreases the anode current continuously as a function of ODN concentration (inset B of Figure 1) until a constant value is reached after 600 nmol of ODN is added. From this curve we can read the concentration of the complementary ODN in solution.

The sensitivity of this electrochemical process was evaluated to 1 μA/nmol of complementary ODN from the slope at the origin of this curve. This sensitivity is a marked improvement when compared to other electrochemical DNA sensors based on intercalators as a hybridization indicator process, exhibiting a sensitivity of 0.05 μA/100 nmole of DNA in solution.[25] The detection limit for this system is 10^{-11} mol of ODN in solution. Compared to the detection limit of 10^{-9} mol achieved with biosensors based on a fluorescent probe,[26] the value of 10^{-11} mol obtained in this work together with its simple electrical reading technique appears very promising.

To analyze if this recognition process can be generalized to longer base sequences, a 25-mer oligonucleotide bearing the sequence $^{5'}$TCA ATC TCG GGA ATC TCA ATG TTA G$^{3'}$ has been grafted on the polypyrrole precursor film. The behavior of this electrode has been studied, following hybridization with noncomplementary and complementary 25-mer oligonucleotide targets. A first incubation of the electrode in a solution of noncomplementary oligonucleotide showed no modification of the electrochemical signal, as could be expected for a nonspecific target. On the other hand, when in the presence of the complementary 25-mer target $^{5'}$CTA ACA TTG AGA TTC CCG AGA TTG A$^{3'}$, the oxidation potential of the 25-mer electrode shows an even higher shift to more positive potential values than that observed with the previous 15-mer electrode in the presence of the 14-mer target. Such increase in oxidation potential shift can be interpreted by a larger interacting sequence in the grafted 25-mer oligonucleotide substituent on the polypyrrole chains. The increased bulkiness and stiffness induced in the pendent groups lead thus to a higher oxidation potential of the conjugated polymer chains.

This latter result brings an interesting perpective for oligonucleotide-functionalized polypyrrole electrodes, as the sensitivity of oligonucleotide recognition appears to increase with the length of interacting complementary base sequences. A further improvement in sensitivity can also be expected from a similar device in a transistor configuration, which gives hopes to reach sensitivity required for pratical applications.

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(27) The precursor copolymer I was electropolymerized at 0.9 V/SCE onto a platinum electrode (0.7 cm^2) in acetonitrile solution containing both monomers, 0.06 M 3-acetic acid pyrrole (II) and 0.04 M 3-*N*-hydroxyphthalimide pyrrole (III), see eq 1. An electropolymerization charge of 70 mC led to a film of about 200 nm thickness, containing 300 nmol of total pyrrole units, assuming a 100% polymerization yield. We verified that this 3:2 ratio of monomers II and III was retained in the distribution of monomer units in the precursor polymer I.