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Label-Free DNA Hybridization Probe Based on a Conducting Polymer

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Herein we report on a new approach to a simple and direct electrochemical detection of a hybridization event which utilizes electrostatic modulation of ion-exchange kinetics of polypyrrole (PPy) film. This ion exchange is controlled by the reaction of a surface immobilized, unlabeled, single-stranded DNA oligomer molecule, ssDNA(a), with its complementary DNA(c). The oligonucleotide is linked to the conducting polymer by forming a bidentate complex between Mg^{2+} and an alkyl phosphonic acid group on the polymer and the phosphate group of the DNA, Figure 1.

The detection of DNA hybridization and damage by electrochemical procedures has been reviewed.[1,2] The immobilization of DNA on electrodes modified with conducting polymers has been previously accomplished by using two approaches. The first approach involves a direct adsorption of ssDNA onto oxidized PPy films.[2,3] In that case, the delocalized positive charge of the oxidized polymer electrostatically attracts the negatively charged phosphate groups of the DNA. The level of adsorption depends on the nature of the anion dopant (electrolyte), solution pH, type, and the ionic strength of the buffer used, and on the DNA itself. That approach precludes recording of the entire cyclic voltammogram, CV, due to desorption of the DNA probe during the reduction half of the cycle. Even when using a different detection method, the orientation of the oligonucleotide probe is difficult to control; the probe is likely to align itself along the surface of the electrode, rather than be extended into solution. The second approach involves incorporation of oligonucleotides *into* the polymer matrix during the growth of the conducting polymer[4,5] or during the copolymerization of the pyrrole monomer with the pyrrole monomers functionalized with the oligonucleotide.[6−11] The containment of the oligonucleotide within the polymer bulk leads to a more permanent immobilization, but it invariably leads to steric and kinetic barriers to the hybridization of a macromolecule. In addition, the oligonucleotide probe is oxidatively damaged by radical cations formed during pyrrole polymerization, leading to its partial degradation. Despite these problems, this approach retains some ability to recognize complementary single strands of DNA and provides the basis for DNA detection.

Early work performed on the polymer allowed for the observation of PPy's strong electrostatic attraction of the oligonucleotide probe. The adsorption of the probe onto the PPy surface caused suppression of current, as observed by the CV. It is surmised that the presence of the oligonucleotide probe at the PPy surface hinders the passage of chloride ions at the PPy/solution interface. However, for a physisorbed probe DNA, further hybridization was electrochemically inconsequential in comparison with the first adsorption step because the complementary DNA only displaced the original DNA without changing the surface charge density.

Our rationale for voltammetric observation of the hybridization event is based on the modulation of ion-exchange characteristics of PPy. This approach takes advantage of the reversible electrochemical behavior of PPy after its surface has been modified with a layer of electrochemically grafted poly(2,5-dithienylpyrrole) modified with a phosphonic acid group, pTPTC3-PO_3H_2 (Figure 1). The binding of the DNA probe via its phosphoric acid residues occurs with the help of magnesium cations. Hereinafter, the Mg^{2+} cation serves as a bridging medium between the phosphonic acid group of the grafted polymer and the phosphate group of the oligonucleotide probe.[12] This type of linkage makes the oligonucleotide offset from the surface of the polymer, giving it some freedom of movement and easing the effect of steric hindrances on the hybridization event. Second, from the structural point of view, the pTPTC3-PO_3H_2 serves as a compatible layer on top of polypyrrole. Third, it is likely that grafting of the poly[2,5-dithienyl-(*N*-3-diethylphosphorylpropyl)pyrrole], pTPTC3-PO_3Et_2, results in an orientation in which the backbone of the modifying polymer aligns itself with the PPy, while the alkyl phosphonic ester group extends into solution. The site of polymerization is along the TPT backbone and does not involve the pendant dialkyl phosphonate ester. When oxidizing potential is applied to induce the polymerization, one can conclude that the electron transfer occurs along the backbone. This leaves the pendant alkyl phosphonate ester oriented toward solution rather than toward the PPy bulk. The dealkylation of the phosphonate esters yields the phosphonic acid groups. The major advantage of this approach is that the immobilization of the unlabeled probe molecule, ssDNA(a), at the surface of the modified electrode is done by a simple dipping. Likewise, the hybridization test involves dipping of the DNA(a)-modified electrode in the test DNA (DNA(b) or DNA(c)) solution, followed by running a CV in a chloride-ion-containing buffer. Thus, the preparation of the modified electrodes can be done in a batch mode, and the prepared electrodes can be stored for future use.

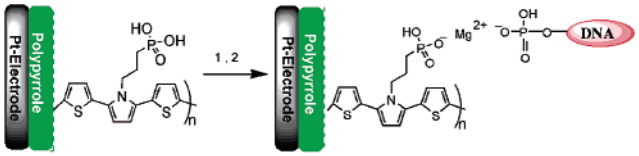

Figure 1. Key steps in the preparation of the DNA hybridization sensor probe: (1) $MgCl_2$ aqueous, 5 mM, 10 min; (2) aqueous ssDNA(a), 10 min, then rinsed with TRIS buffer.

The electrochemically active medium was prepared on a platinum electrode by an initial polymerization of pyrrole followed by grafting a top layer of the pTPTC3-PO_3Et_2. The polymerization was done at a constant potential (0.7 V vs Ag/Ag^+) from acetonitrile solution of tetraethylammonium perchlorate. The modified electrodes were then reduced by applying a short potential step to −0.3 V in the monomer-free electrolyte solution. The presence of the diethyl phosphonate ester groups in the polymer was confirmed by its signature infrared frequencies at 1250 and 1050 cm^{-1} associated with the phosphodiester function.[13] The hydrolysis of the phosphonic ethyl esters was carried out by immersing electrodes covered with the polymer in a solution of iodotrimethylsilane in carbon tetrachloride (3 h, room temperature, in the dark) followed by soaking in methanol.[14] The electrodes containing phosphonic

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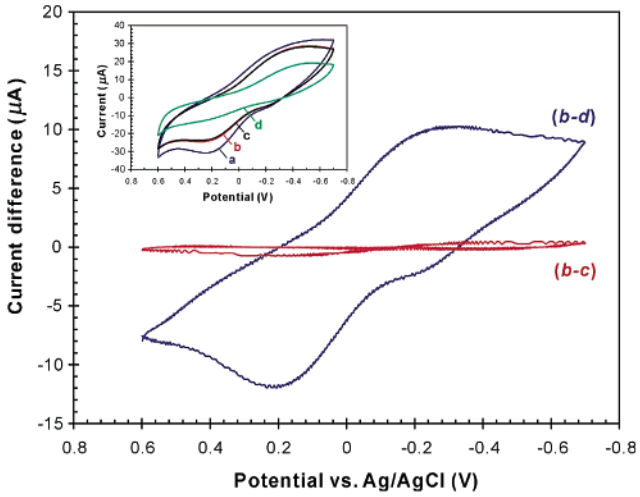

Figure 2. Voltammetric detection of hybridization event. Scan rate was 20 mV s^{-1}, and the electrode area was 0.018 cm^2. Complementary interaction, curve b-d, and noncomplementary interaction, curve b-c. Sequence of events and recorded CVs are shown in the inset: curve a − PPy/pTPTC3−PO_3H_2 after Mg^{2+} adsorption; curve b − same probe as in curve a after adsorption of DNA(a); curve c − same probe as in curve b after exposure to DNA(b); curve d − same probe as curve b after exposure to DNA(c).

acid residues were then soaked in a 5 mM aqueous solution of $MgCl_2$ for 10 min to complete the preparation of a generic "activated" electrode ready for binding the probe DNA.

The specificity of the biorecognition was studied with the synthetic 27-mer strands of oligonucleotide of the sequence as shown below.[15]

DNA	function	base sequence
(a)	probe DNA	5′CGA AAA TGA ATA AAC TAG TAA GGA AGT 3′
(b)	noncomplementary to probe (a)	3′ ACT TCC TTA CTA GTT TAT TCA TTT TCG 5′
(c)	complementary to probe (a)	3′ GCT TTT ACT TAT TTG ATC ATT CCT TCA 5′

The validity of the new procedure was further confirmed with a 39-mer oligonucleotide system.[16] The activated electrode was placed in the ssDNA(a) probe solution for 10 min, followed by rinsing with 0.1 M TRIS−HCl buffer, pH 7.3. The thus modified electrode was placed in a solution of the noncomplementary oligonucleotide, and the CV was recorded (Figure 2, curve c). The electrode was then placed in the solution of the complementary oligonucleotide, and the CV was recorded again (Figure 2, curve d). All cyclic voltammograms were run in a TRIS/HCl buffer (pH 7.3). The uptake (oxidation) and the expulsion (reduction) resulted in a characteristic CV of polypyrrole. The cyclic voltammograms indicated a small decrease in current after the ssDNA(a) was immobilized on the electrode (Figure 2, curve b). Optimally, the sensor should not respond to the presence of an oligonucleotide possessing a noncomplementary sequence. The recognition layer showed no effect on CV when it was exposed to the solution containing a noncomplementary oligonucleotide DNA(b) (Figure 2, curve c). However, when a complementary oligonucleotide sequence DNA(c) was introduced and hybridization took place, there was a marked change of the shape of CV. These effects are best seen in the difference CVs, shown in Figure 2; (b-d) and (b-c) relate to complementary and noncomplementary interactions, respectively.

In summary, our results indicate that the addition of negative charge to the surface of the electrode, in the form of complementary oligonucleotide, further hinders the chloride ion exchange as seen from the decrease of CV current. Thus, noncomplementary and complementary interactions can be clearly distinguished.

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References

(1) Palecek, E.; Fojta, M. *Anal. Chem.* **2001**, *73*, 74A−83A.
(2) Palecek, E. *Talanta* **2002**, *56*, 809−819.
(3) Minehan, D. S.; Marx, K. A.; Tripathy, S. K. *Macromolecules* **1994**, *27*, 777−783.
(4) Saoudi, B.; Jamul, N.; Abel, M.-L.; Chehimi, M. M.; Dodin, G. *Synth. Met.* **1997**, *87*, 97−103.
(5) Wang, J.; Jiang, M.; Fortes, A.; Mukherjee, B. *Anal. Chim. Acta* **1999**, *402*, 7−12.
(6) Wang, J.; Jiang, M. *Langmuir* **2000**, *16*, 2269−2274.
(7) Livache, T.; Roget, A.; Dejean, E.; Barthet, C.; Bidan, G.; Teoule, R. *Synth. Met.* **1995**, *71*, 2143−2146.
(8) Lassalle, N.; Vieil, E.; Correira, J. P.; Abrantes, L. M. *Synth. Met.* **2001**, *119*, 407−408.
(9) Korri-Youssoufi, H.; Garnier, P.; Srivastava, P.; Godillot, P.; Yassar, A. *J. Am. Chem. Soc.* **1997**, *119*, 7388−7389.
(10) Garnier, P.; Korri-Youssoufi, H.; Srivastava, P.; Mandrand, B.; Delair, T. *Synth. Met.* **1999**, *100*, 89−94.
(11) Korri-Youssoufi, H.; Yassar, A. *Biomacromolecules* **2001**, *2*, 58−64.
(12) Koutsodimou, A.; Kovala-Demertzi, D.; Katsaros, N. *J. Coord. Chem.* **1998**, *43*, 1−12.
(13) *Spectrometric Identification of Organic Compounds*, 6th ed.; Silverstein, R. M., Webster, F. X., Eds.; John Wiley & Sons: New York, 1998; p 142.
(14) Blackburn, G. M.; Ingleson, D. *Chem. Commun.* **1978**, 870−871.
(15) Purchased from Integrated DNA Technologies.
(16) *Biosens. Bioelectron.*, submitted.

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