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# Novel label-free DNA sensors based on poly(3,4-ethylenedioxythiophene)†

**K. Krishnamoorthy,[a] Rajesh S. Gokhale,[b] Aliasgar Q. Contractor[a] and Anil Kumar*[a]**

[a] *Chemistry Department, Indian Institute of Technology Bombay, Mumbai 400076, India. E-mail: anilkumar@iitb.ac.in*

[b] *National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India*

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**We report on the design and development of a novel label-free DNA sensor based on conducting poly(3,4-ethylenedioxythiophene) for the direct detection and quantification of target ssDNA.**

In this age of molecular genetics a lot of research efforts are directed towards the understanding of DNA and RNA sequences in order to diagnose, prevent, and treat many human diseases. Simple, economical and efficient detection of various nucleotides sequences is also of prime importance for applications such as forensic medicine, rapid detection of biological warfare agents, and environmental testing.[1] The basic principle for the detection of DNA relies on the detection of duplex formation between a single strand DNA (ssDNA), which is part of the sensor, with that of the target DNA having a sequence that is exactly complementary.[2]

In an electrochemical DNA hybridization detector, a short ssDNA is usually immobilized on a transducer to create a DNA recognition element. Hybrid formation is then translated into an electrical, analytically useful signal. Formation of the DNA recognition layer and the ability to detect the duplex formation determines the specificity and the sensitivity of the device. Conjugated polymer matrices offer extraordinary potential to act as transducers for detecting such duplex formation because the electrical, optical, and electrochemical properties are strongly affected by relatively small perturbations. As a result of this sensitivity, conjugated polymers have been used for various chemical and biological sensors.[3] The main advantages of these devices are their low-cost, simple design, small dimensions, and low power requirements. Two distinct approaches have been used for the immobilization of ssDNA into conducting polymer matrices. The first approach is based on the electrostatic interaction wherein the ssDNA was immobilized after the polymerization by keeping the polymer in an oxidized state.[4,5] This restricts the amount of loading and the probe leaches out during the meaurement which then results in poor sensitivities. The second approach is based on the incorporation of ssDNA during the polymerization. This can be achieved either by the physical entrapment of ssDNA during the electropolymerization[6] or by using a monomer containing ssDNA as a side chain.[7] Although more stable and sensitive DNA sensors can be fabricated using the ssDNA modified monomers, synthetic difficulties often restrict its potential.

In principle, DNA sensors based on the direct physical entrapment of the ssDNA into the conjugated polymer matrix have the advantage of ease of synthesis and can be used as label-free detection of target DNA. Therefore, this method has the potential for commercialization, provided the specificity and the sensitivity are significant. Interestingly, there is only one report in the literature where an attempt has been made in this direction using polypyrrole films as transducers, wherein oligonucleotides containing either adenine or guanine acted as probes.[6] Although the sensor response was found to be linear when adenine was used as a probe, the change in currents was very small (from 1 nA to 4 nA) along with a very small linear range (1–7 $\times$ $10^{-6}$ g). The linear response was even smaller for a guanine probe. Surprisingly, these sensors responded to non-complementary targets as well and this has been attributed to the redox behaviour of polypyrrole in the presence of different counter ions. It is clear from the above discusssion that a label-free DNA sensor based on a conducting polymer which is specific to the complementary target and detect various concentrations with a linear range has not yet been reported.

Here we report on the development of a novel design for the fabrication of biosensors for DNA detection. The present sensor has many salient features such as: no labelling or modification of the ssDNA or the transducing matrix, ease of fabrication, better sensitivity, higher detection range, detection of small segments in a longer oligonucleotide and faster response time. In order to fabricate a DNA sensor, PEDOT was used as an immobilization matrix-cum-physicochemical transducer because PEDOT is highly stable, can be synthesized and used at physiological pH and its conductivity does not change significantly with changes in counter ions.[8] The concept of the present sensor is based on the change in the conformation of the polymer due to the formation of a DNA duplex. This is manifested macroscopically in terms of changes in the conductivity of the polymer. This concept has been explored by us successfully in the past for the design and fabrication of various sensors.[9]

PEDOT microtubules were synthesized from EDOT (0.01 M) and 0.1 M KCl dissolved in pH 8 phosphate buffer in the pores of a gold coated polycarbonate membrane by cycling the potential between $-0.2$ V and 1.3 V *vs.* SCE with a scan rate of 50 mV $s^{-1}$.[10] Polymerization started at one side of the electrode, grew through the pore and reached the other side of the pore. This eventually connects the two gold electrodes on the opposite sides of the membrane. The PEDOT microtubules were found to be stable in pH 8 phosphate buffer solution. The DNA sensors were fabricated by electropolymerization from EDOT solution as mentioned above but in the presence of probe ssDNA. In order to study the effect of ssDNA length, sensors with 20mer, 10mer and 5mer ssDNA (Table 1) were prepared. Irrespective of the number of bases in the oligonucleotide probe the redox behavior of PEDOT remained the same. The sensor measurements were carried out by exposing the device to various concentrations of complementary ssDNA in pH 8 phosphate buffer solution. The response is represented by $\Delta R/R_0$ where $\Delta R = R - R_0$; $R_0$ is the resistance of the sensor without any substrate and $R$ is the resistance of the sensor in the presence of the substrate. The response of the devices was measured following the reported experimental technique.[10] The sensor prepared with 20 μg $ml^{-1}$ of ssDNA (20mer A) was exposed to various concentrations of complementary ssDNA (A1). The sensor measurements were

**Table 1** Oligonucleotide sequences

| ssDNA | Sequence |
|---|---|
| **A** | 5′-GGACATGTTGCCGAAGCG GG-3′ |
| **B** | 3′-GGGCGAAGCC-5′ |
| **C** | 5′-CCC GC-3′ |
| **A1** | 3′CCTGTACAACGGCTTCGCCC-5′ |
| **B1** | 5′-CCC GCT TCG G-3′ |
| **C1** | 3′-GGG CG-5′ |

† Electronic Supplementary Information (ESI) available: Experimental details and sensor response for various sensors listed in Table 2. Cyclic voltammograms confirming the increase in resistance due to the duplex formation between the probe and target DNA in conducting polymer matrix. See http://www.rsc.org/suppdata/cc/b3/b316794a/

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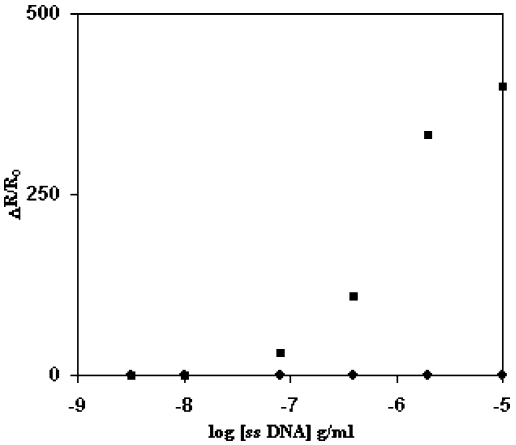


**Fig. 1** Sensor response as a function of ssDNA concentration (A1). ■, Complementary strand; ◆, control experiment (PEDOT device fabricated in the absence of ssDNA probe).

done at a gate potential of +0.8 V because the sensor response was found to be highest at this potential. The linear range was observed from $8 \times 10^{-8}$ to $1 \times 10^{-5}$ g ml$^{-1}$ (Fig. 1). In order to prove that the sensor response is due to the DNA duplex formation, a control experiment was done using PEDOT synthesized in the absence of oligonucleotide probe. This sensor showed negligible response when it was exposed to various concentrations of ssDNA (A1) (Fig. 1). In order to prove that the sensor response is a result of the duplex formation between the probe and the target ssDNA in the conducting polymer, the electrode was kept in ethidium bromide for 30 min after the sensor measurement. The same experiment was done for the control experiment as well. Both the electrodes were taken from ethidium bromide and kept in Tris–EDTA solution for 1 h. Fluorescence spectra were recorded by exciting the solution at 254 nm. The solution obtained from the electrode containing the immobilized probe ssDNA emits at 314 and 372 nm (Fig. 2). However, the solution from the control experiment emits at the same wavelength but the peaks are very weak. From this experiment it can be concluded that the DNA duplex has formed in the conducting polymer between the probe and the complementary ssDNA.

In order to study the effect of ssDNA length, sensors were fabricated using 20 μg ml$^{-1}$ of 10mer of ssDNA (B). This sensor was exposed to various concentrations of complementary ssDNA (B1) and the linear range was observed from $6 \times 10^{-7}$ to $1 \times 10^{-5}$ g ml$^{-1}$ (Table 2). Note that the linear range as well as the sensor response (450 times) was found to be more in the case of 20mer, which is due to the large conformational changes upon duplex

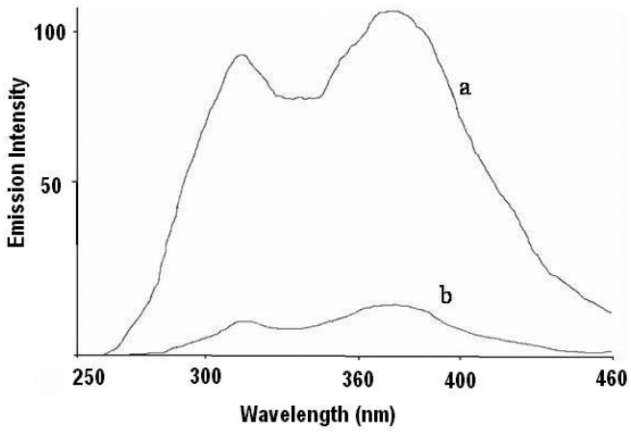


**Fig. 2** Fluroscence spectra of (a) ethidium bromide intercalated dsDNA and (b) a control experiment where no dsDNA was expected (see text for details).

**Table 2** Detection range for various sensors

| Probe ssDNA | Conc.[a]/ μg ml$^{-1}$ | Target ssDNA | Detection range/g ml$^{-1}$ |
|---|---|---|---|
| A (20mer) | 20 | A1 (20mer) | $80 \times 10^{-9}$–$0.01 \times 10^{-3}$ |
| B (10mer) | 20 | B1 (10mer) | $0.6 \times 10^{-6}$–$0.01 \times 10^{-3}$ |
| B (10mer) | 20 | A (20mer) | — |
| C1 (5mer) | 10 | B1 (10mer) | $0.9 \times 10^{-6}$–$6 \times 10^{-6}$ |
| C1 (5mer) | 10 | B (10mer) | — |
| B (10mer) | 10 | C (5mer) | $0.5 \times 10^{-6}$–$3 \times 10^{-6}$ |

[a] Concentration in the polymerization solutions.

formation. Furthermore, the sensor based on 10mer ssDNA (B) was exposed to various concentrations of noncomplementary 20mer strand (A) in order to confirm the specificity. No significant changes were observed in sensor response indicating that the sensor response is specific to the complementary strand. The true potential of these sensors will be if a smaller probe strand can be used to detect a longer target strand having the complementary sequence anywhere along the length and *vice versa*. The sensor response for these types of devices is also listed in Table 2. In these experiments the resistance of the film has increased upon increasing the concentration of the complementary strand in the solution. The increase in resistance of the film upon duplex formation was confirmed by cyclic voltammetry where a decrease in redox charge was observed upon duplex formation.

In conclusion, we have shown that the present DNA sensor is novel in many respects such as: a) this is a label-free DNA sensor which can be fabricated easily; b) it can detect concentrations as low as $80 \times 10^{-9}$ g ml$^{-1}$; c) a transducer immobilized with a 5mer can be used to detect a longer strand. The lower limit for the detection can be further improved by increasing the loading of the probe ssDNA and work is in progress in this direction.

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