Cationic Polythiophene for Label-Free, Colorimetric and Fluorometric Detection of DNA

H.A. Ho* and M. Leclerc*

*Département de chimie, Université Laval, Québec City, Qc, G1K 7P4, Canada, hoang-anh.ho@chm.ulaval.ca

ABSTRACT

In order to respond to new demands and needs in modern medical diagnostics and biomedical research applications, a lot of new DNA detection systems have been reported [1-7]. However, most of these methods have the disadvantage of requiring the chemical coupling of a photoactive or electroactive tagging agent onto the target or the probe prior to detection. Some oligonucleotide-functionalized conjugated polymers have enabled the transduction of hybridization events, without labeling of the DNA target [8-10]. Here we describe the use of a water-soluble, cationic polythiophene that can specifically transduce the binding of an appropriate oligonucleotide to its target into a clear optical (colorimetric or fluorometric) signal. This simple, rapid, sensitive and selective methodology does not require any chemical modification on the probes or targets.

Keywords: conjugated polymer, DNA recognition, electrostatic interaction, sensors, fluorescence.

1 EXPERIMENTAL SECTION

1.1 Synthesis

Polymeric transducer, poly(1H-imidazolium, 1-methyl-3-[2-[(4-methyl-3-thienyl)oxy]ethyl]-, chloride), was prepared following already published procedures [11-12] (Figure 1).

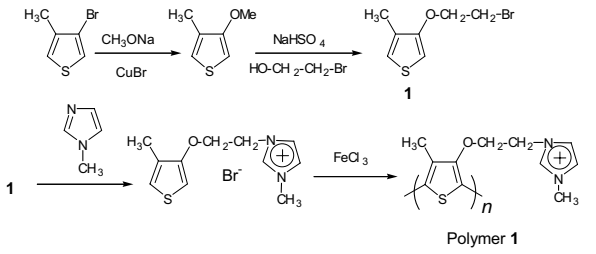

Figure 1: Synthesis of polymer **1**.

1.2 DNA Hybridization

All oligonucleotide solutions were prepared using sterilized 0.1 M NaCl aqueous solutions. In a quartz cuvette with an optical pathlength of 1.0 cm, a 13.4 µL aliquot of a 7.2 x 10^{-4} M (on a repeat unit basis) aqueous solution of polymer was added to 100 µL of an aqueous solution of either 0.1M NaCl or 10 mM Tris buffer containing 0.1M NaCl (pH 8). This mixture was heated at 55°C for 5 min, followed by the addition of 4 µL of a 1.2 x 10^{-4} M solution of the capture oligonucleotide (20 nucleic acids), and the resulting red solution was kept at 55°C for 5 more minutes. The appropriate oligonucleotide target (volume of 4 µL) was added to the solution at 55°C over 5 minutes.

2 DNA DETECTION

2.1 Colorimetric Method

The aqueous solution of the cationic, conjugated polymer is yellow (λ_{max}= 397 nm) (Figure 3A,a and 3B,a). This absorption maximum at a relatively short wavelength is related to a random coil conformation of the polythiophene derivative, any twisting of the conjugated backbone leading to a decrease of the effective conjugation length (Figure 2). As with any water-soluble cationic polyelectrolytes, this polythiophene derivative can make strong complexe with negatively-charged oligomers and polymers. For instance, we report here the utilization of three types of negatively-charged oligonucleotides: a capture probe sequence (**X1**: 5'-CATGATTGAACCATCCACCA-3'), a perfect complementary target (**Y1:** 3'-GTACTAACTTGGTAGGTGGT-5'), a two-mismatch complementary target (**Y2:**3'-GTACTAACTTCGAAGGTGGT-5') and an one-mismatch complementary target (**Y3:** 3'-GTACTAACTTCGTAGGTGGT-5'). Upon addition of 1 equivalent, on a monomer unit basis of capture oligonucleotide X1, the mixture becomes red (λ_{max}= 527 nm) (Figure 3A,b and 3B,b) because of the formation of a so-called duplex between the polythiophene and the oligonucleotide probe (Figure 2). After 5 minutes of mixing in the presence of 1 equivalent of the complementary

oligonucleotide Y1, the solution becomes yellow (λ_{max}= 421nm) (Figure 3A,c and 3B,c); presumably caused by the formation of a new complex termed a triplex (Figure 2), formed by complexation of the polymer with the hybridized nucleic acids.

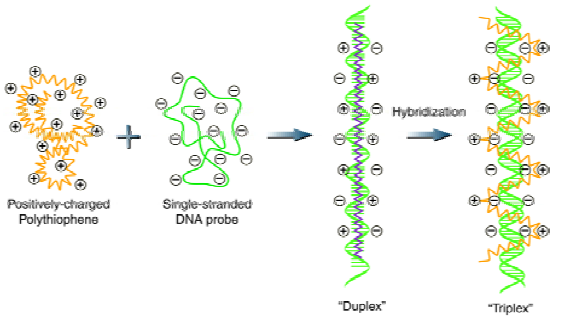

Figure 2: Schematic description of the formation of the polythiophene/single-stranded nucleic acid duplex and the polythiophene/hybridized nucleic acid triplex.

In order to verify the specificity of this polymeric optical transducer in the presence of imperfect or incomplete hybridizations, two different 20-mers oligonucleotides differing by only 2 or 1 nucleotides were investigated. A very distinct, stable and reproducible UV-visible absorption spectrum is observed in the case of oligonucleotide target having two mismatches Y2 (Figure 3B, d) when compared to perfect hybridization (Figures 3B curve c). It is even possible to distinguish only one mismatch (Figures 3A,e and 3B,e).

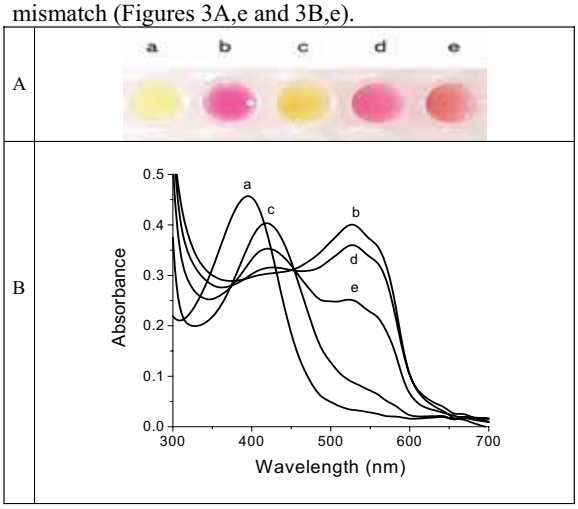

Figure 3:Photographs of 7.9 x 10^{-5} M (on a monomeric unit basis) solutions of a) polymer, b) polymer / **X1** duplex, c) polymer / **X1**/ **Y1** triplex, d) polymer / **X1**/ **Y2** mixture, and e) polymer / **X1**/ **Y3** mixture after 5 minutes of mixing at 55 ^{0}C in 0.1 M NaCl/H_2O. B) UV-visible absorption spectra corresponding to the different assays of photograph A. (Reprinted with permission from reference 11).

The detection limit of this colorimetric method is about 1x10^{13} molecules of oligonucleotide (20-mers), in a total volume of 100 µL (which gives a concentration of 2x10^{-7} M).

2.2 Fluorometric Method

A fluorometric detection of oligonucleotide hybridization is also possible since the fluorescence of poly(3-alkoxy-4-methylthiophene)s is quenched in the planar, aggregated form. For instance, at 55 ^{0}C, the yellow form of polymer **1** is fluorescent (quantum yield of 0.03 with a maximum of emission at 530 nm, see Figure 4, a) but upon addition of 1.0 equivalent of a negatively-charged capture oligonucleotide probe **X1**, the fluorescence intensity decreases and the maximum of emission is slightly red-shifted (Figure 4, b). When hybridization with the complementary strand **Y1** takes place, the formation of a polymeric triplex leads to a 5-fold rise in fluorescence intensity (Figure 4, c). Interestingly, upon addition of 1 (Figure 4, d) or even 100 equivalents (Figure 4, d') of the target oligonucleotide with two mismatches **Y2**, the fluorescence intensity is not significantly modified. It is even possible to distinguish oligonucleotides with one mismatch (Figure 4 curve e).

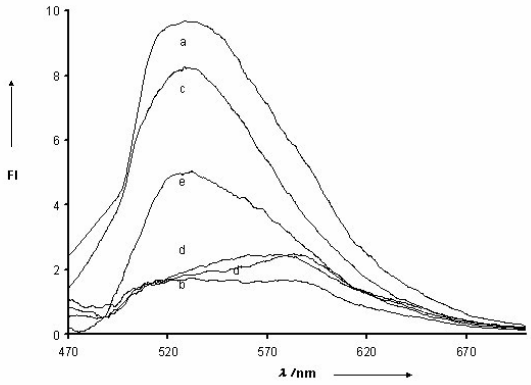

Figure 4: Fluorescence spectrum of a 2.0 x 10-7 M (on a monomeric unit basis) solution of a) poly 1, b) poly 1 /X1 duplex, c) poly 1 /X1 /Y1 triplex, d) poly 1/X1 / Y2 mixture, d') poly 1/X1/Y2 (100 equivalents) mixture and e) poly 1/X1/Y3 mixture at 55°C. (Reprinted with permission from reference 11).

By measuring the fluorescence intensity at 530 nm (without recording the entire emission spectrum), it is possible to detect the presence of as few as 3x10^6 molecules of the perfect complementary oligonucleotide (20-mers) in a volume of 200µL (this is a concentration of 2x10^{-14}M). Moreover, by using a custom fluorometer based on a high-intensity blue diode (as the excitation source) and a nondispersive, interference filter, a few hundred copies of either DNA or RNA can be specifically detected [12]. For example, in the case of 20-mer target oligonucleotides, a limit of detection of 310 molecules or 0.54x10^{-21} mol in an

effective volume of 150μL, or $3.6x10^{-18}$M was obtained. In comparison with the perfect hybridization, the presence of sequences having one or two mismatches induces only a slight increase of the luminescence intensity and the addition of a large excess (100 equivalents) of the oligonucleotide with two mismatches only leads to a moderate increase of the luminescence.

In conclusion, a novel methodology that allows simple optical (colorimetric or fluorometric) detection of nucleic acids has been developed. This rapid, selective, sensitive and versatile method does not require any chemical modification of the probes or the analytes and is based on conformational changes of the conjugated backbone of cationic polythiophene, when mixed with single-stranded or double-stranded (hybridized) oligonucleotides. This procedure could provide inexpensive systems for the rapid detection and identification of nucleic acids.

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