J|A|C|S
ARTICLES
Published on Web 01/15/2004

Optical Sensors Based on Hybrid Aptamer/Conjugated Polymer Complexes

Hoang-Anh Ho and Mario Leclerc*

Contribution from the Canada Research Chair in Electroactive and Photoactive Polymers, Centre de recherche en sciences et ingénierie des macromolécules, Département de chimie, Université Laval, Quebec City, Quebec, Canada G1K 7P4

Received July 15, 2003; E-mail: Mario.Leclerc@chm.ulaval.ca

Abstract: Single-stranded DNA (aptamer) can specifically bind potassium ions or human α-thrombin. When binding takes place, the aptamer undergoes a conformational transition from an unfolded to a folded structure. This conformational change of the negatively charged oligonucleotide can be detected by adding a water-soluble, cationic polythiophene derivative, which transduces the new complex formation into an optical (colorimetric or fluorometric) signal without any labeling of the probe or of the target. This simple and rapid methodology has enabled the detection of human thrombin in the femtomole range. This new biophotonic tool can easily be applied to the detection of various other proteins as well as being useful in the high-throughput screening of new drugs.

Introduction

Intense research activities are carried out worldwide to develop rapid, simple, specific, and sensitive detection tools for medical diagnostics and biomedical research applications. Fundamentally, most analytical tests and immunoassays rely on molecular recognition and its transduction into a measurable output. Among all possible molecular recognition elements, artificial nucleic acid ligands (aptamers) have recently attracted a lot of interest due to their capability to bind various metal ions, amino acids, drugs, proteins, and other molecules with high affinity and specificity.[1−11] Aptamers are usually isolated from combinatorial libraries of synthetic nucleic acids by an iterative process of adsorption, recovery, and amplification (coined as SELEX for systematic evolution of ligands by exponential procedure). Aptamer-based ligands are certainly highly promising candidates for the specific detection of various molecules but can also be used in competition binding assays to identify compounds that are capable of displacing the aptamers from their targets for high-throughput screening assays.[7] However, as mentioned above, all these approaches also require adequate transducing (i.e., reporting) elements to generate a physically measurable signal from the recognition event. For example, binding of an aptamer to a target protein has been detected by using fluorescence (e.g., molecular beacons[12,13]) or quartz microbalance[14]. However, in most cases, these methods involve a tagging process or sophisticated experimental techniques. It is worth noting that labeling with various functional groups may even compromise the binding properties of the aptamers.

In this respect, we describe here the use of a water-soluble cationic polythiophene as a "polymeric stain" that can specifically transduce the binding of an aptamer 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 the analytes and is based on different electrostatic interactions and conformational structures between a cationic poly(3-alkoxy-4-methylthiophene) derivative and anionic single-stranded oligonucleotides. For instance, this method can specifically detect as little as 2×10^{-15} mol of human thrombin in few minutes and could be easily adapted for many other chemical or biochemical targets.

Results and Discussion

The cationic, water-soluble, electroactive, and photoactive polymer **1** (Figure 1) was synthesized according to recently published procedures.[15] As most poly(3-alkoxy-4-methylthiophene)s,[16−19] polymer **1** exhibits chromic properties (color changes) due to conformational changes of the flexible conjugated backbone. Moreover, polymer **1** is known to display

(1) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505.
(2) Robertson, M. P.; Joyce, G. F. *Nature* **1990**, *344*, 467.
(3) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818.
(4) O'Sullivan, C. K. *Anal. Bioanal. Chem.* **2002**, *372*, 44.
(5) Clark, S. L.; Remcho, V. T. *Electrophoresis* **2002**, *23*, 1335.
(6) Drolet, D. W.; McDermott, L. M.; Romig, T. S. *Nat. Biotechnol.* **1996**, *14*, 1021.
(7) Green, L. S.; Bell, C.; Janjic, N. *Biotechniques* **2001**, *30*, 1094.
(8) Davis, K. A.; Abrams, B.; Lin, Y.; Jayasena, S. D. *Nucleic Acids Res.* **1996**, *24*, 702.
(9) Lin, Y.; Nieulandt, D.; Magallanez, A.; Feistner, B.; Jayasena, S. D. *Nucleic Acids Res.* **1996**, *24*, 3407.
(10) Blank, M.; Weinschenk, T.; Priemer, M.; Schluesener, H. *J. Biol. Chem.* **2001**, *276*, 16464.
(11) Lee, M.; Walt, D. R. *Anal. Biochem.* **2000**, *282*, 142.
(12) Hamaguchi, N.; Ellington, A.; Stanton, M. *Anal. Biochem.* **2001**, *294*, 126.
(13) Jianwei, J. L.; Xiaohong, F.; Weihong, T. *Biochem. Biophys. Res. Commun.* **2002**, *292*, 31.
(14) Liss, M.; Petersen, B.; Wolf, H.; Prohaska, E. *Anal. Chem.* **2002**, *74*, 4488.
(15) Ho, H. A.; Boissinot, M.; Bergeron, M. G.; Corbeil, G.; Doré, K.; Boudreau, D.; Leclerc, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1548.
(16) Leclerc, M. *Adv. Mater.* **1999**, *11*, 1491.
(17) Faïd, K.; Leclerc, M. *J. Am. Chem. Soc.* **1998**, *120*, 5274.
(18) Bernier, S.; Garreau, S.; Béra-Abérem, M.; Gravel, C.; Leclerc, M. *J. Am. Chem. Soc.* **2002**, *124*, 12463.
(19) Ho, H. A.; Leclerc,M. *J. Am. Chem. Soc.* **2003**, *125*, 4412.

10.1021/ja037289f CCC: $27.50

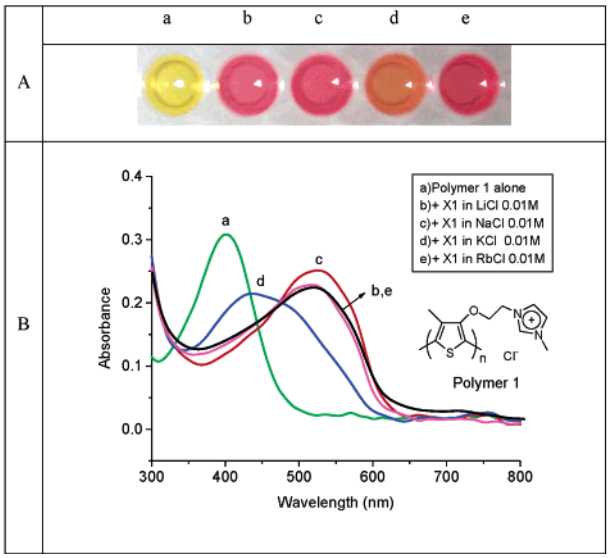

Figure 1. (A) Photographs and (B) UV−vis absorption spectra of polymer **1** (2.9×10^{-9} mol on a monomer unit basis) in the presence of X1 (1.9×10^{-10} mol of the 15-mer) and different salts in 100 μL of water, at 25 °C.

important optical changes when complexed to ssDNA or dsDNA[15] that makes it a promising candidate to transduce binding of an aptamer to a given target. The monovalent potassium cation was first selected because of its known folding-inducing properties for several classes of nucleic acids.[20,21] As shown in Figure 1, an aqueous solution of polymer **1** is yellow with a maximum of absorption (λ_{max}) at 402 nm (illustrations a in both panels of Figure 1).

This absorption maximum at a relatively short wavelength should be related to a random-coil conformation of the polythiophene derivative, as any twisting of the conjugated backbone leads to a decrease of the effective conjugation length.[16] A red color (λ_{max} = 527 nm) was observed in the presence of LiCl (illustrations b in both panels of Figure 1), NaCl (illustrations c in both panels of Figure 1), or RbCl (illustrations e in both panels of Figure 1) and ss-DNA (X1, 5′-GGTTGGTGTGGTTGG-3′). This red shift is related to a stoichiometric complexation between unfolded anionic ss-DNA and the cationic polythiophene derivative (Figure 2, path A). Such stoichiometric polyelectrolyte complexes tend to be insoluble in the medium in which they are formed.[15] These red-violet (probably formed from planar polymer chains) aggregates show an absorption spectrum like that obtained in the solid state. However, the optical properties (illustrations d in both panels of Figure 1) are different when potassium ions are present. The formation of quadruplex state of oligonucleotide X1 stabilized by K^+ allows polymer **1** to wrap this folded structure (Figure 2, path B) through electrostatic interactions. Moreover, similar results were observed when the chloride counterion was replaced by bromide or iodide anion, indicating the specificity of the detection toward potassium cations.

As a second example, the human α-thrombin was selected since X1 (5′-GGTTGGTGTGGTTGG-3′) is also known to be a specific binding sequence (i.e., an aptamer) for this protein, whereas another oligonucleotide (X2, 5′-GGTGGTGGTTGTGGT-3′) is known to be a nonbinding sequence.[22] A conformational change occurs when the aptamer binds to the thrombin molecule. Both NMR[23] and X-ray diffraction studies[24] have revealed that the aptamer adopts a compact unimolecular quadruplex structure with two G-quartets. Therefore, as shown in Figure 3, the specific detection of human α-thrombin could be realized due to the formation of a quadruplex structure of the aptamer (X1).

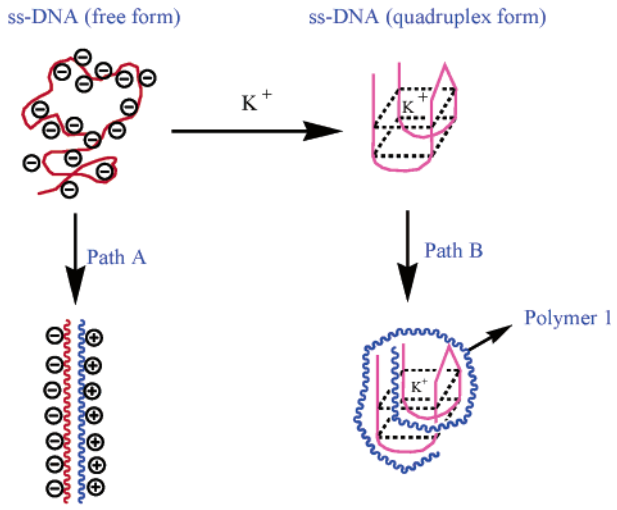

Figure 2. Principle of specific detection of potassium ions.

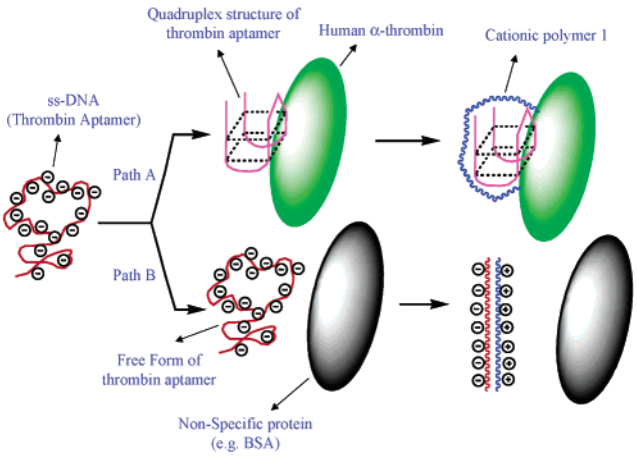

Figure 3. Schematic description of the specific detection of human α-thrombin by use of ss-DNA thrombin aptamer and cationic polymer **1**.

Accordingly, the 1:1:1 complex between polymer **1**, oligonucleotide X1, and thrombin has the same orange color and UV−vis absorption spectrum (Figure 4, spectrum b) as that induced by K^+. The thrombin promotes the formation of quadruplex form of thrombin aptamer and the cationic polymer **1** wraps this quadruplex structure, which seems to partially hinder the aggregation and planarization of the positively charged polymer **1** in the presence of ssDNA X1 (Figure 3, path A). It is worth noting that only the stoichiometry of the aptamer (in terms of negative charges) and of polymer **1** (in terms of positive charges) has to be balanced, whereas an excess of thrombin does not influence its detection. To verify the specificity of the detection, two control experiments with a

(20) Basu, S.; Szewczak, A. A.; Cocco, M.; Strobel, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 3240.
(21) Ueyama, H.; Takagi, M.; Takenaka, S. *J. Am. Chem. Soc.* **2002**, *124*, 14286.
(22) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564.
(23) Wang, K. Y.; McCurdy, S.; Shea, R. G.; Swaminathan, S.; Bolton, P. H. *Biochemistry* **1993**, *32*, 1899.
(24) Padmanabhan, K.; Padmanablan, K. P.; Ferrara, J. D.; Sadler, J. E.; Tulinsky, A. *J. Biol. Chem.* **1993**, *268*, 17651.

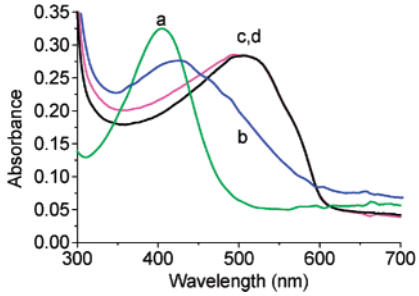

Figure 4. UV−vis absorption spectra corresponding to the different assays recorded at 5 °C: (a) polymer 1 alone in water; (b) complex (1/1/1) of human thrombin/specific thrombin DNA aptamer (X1)/polymer **1**; (c) mixture of human thrombin/nonspecific thrombin DNA aptamer (X2)/polymer **1**; (d) mixture of BSA/specific thrombin DNA aptamer (X1)/polymer **1**.

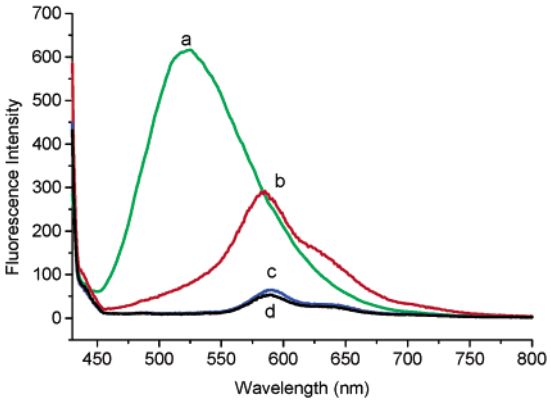

Figure 5. Fluorescence spectra of (a) polymer **1**, (b) human thrombin/X1/polymer **1** complex, (c) human thrombin/X2/polymer **1** mixture, and (d) X1/polymer **1** complex in water, measured at 5 °C.

nonbinding sequence (X2) (Figure 4, spectrum c) and BSA (bovine serum albumin) (Figure 4, spectrum d) were carried out under identical conditions. In both cases, an important red shift toward lower energy (λ_{max} = 505 nm) was observed and the color of these solutions were red-violet, a typical color of the planar and highly conjugated structure of the polythiophene backbone when mixed with unfolded ssDNA (Figure 3, path B). The detection limit by this colorimetric method is about 1 × 10^{-11} mol of thrombin in a total volume of ca. 100 μL (which gives a concentration of about 1 × 10^{-7} M).

Interestingly, the fluorescent properties of anionic or cationic conjugated polymers can be utilized to detect very small quantities of analytes in aqueous solutions.[15−19,25−34] A fluorometric detection of the thrombin aptamer binding to the human α-thrombin is possible because the fluorescence of poly(3-alkoxy-4-methylthiophene) is quenched in the planar, aggregated form.[15−19] The yellow, random-coil form of polymer **1** is fluorescent (quantum yield of 0.03, measured against polythiophene standards[15]) (Figure 5, spectrum a) with an emission maximum at 525 nm.

With use of nonspecific thrombin aptamer (X2) (Figure 5, spectrum c) or the absence of human thrombin (Figure 5, spectrum d), the red-violet, highly conjugated form has a much lower fluorescence intensity and the maximum of emission is red-shifted (λ_{em} = 590 nm). However, when the 1:1:1 complex (human thrombin/specific thrombin DNA aptamer X1/polymer **1**) is formed (Figure 5, spectrum b), the resulting orange intermediate form is less fluorescent than the yellow form but more fluorescent (ca. a 6-fold increase) than the red-violet form. This higher intensity (turn-on) of emission could be related to a partially planar conformation of the polythiophene chain but with less aggregation of the chains.[30] By use of a standard spectrofluorometer, a detection limit of 2 × 10^{-15} mol (this is a concentration of 1 × 10^{-11} M in 200 μL) of the human α-thrombin was obtained. Moreover, an optimized fluorescence detection based on a high-intensity blue diode (as the excitation source) and a nondispersive, interference filter-based system should yield even more sensitive detection capability, and these approaches will be tested soon.

Conclusion

In conclusion, a new selective and highly sensitive method (as few as 2 × 10^{-15} mol) of detection of the human α-thrombin has been developed by using hybrid anionic aptamer/cationic polythiophene complexes. This rapid, specific, sensitive, and versatile approach does not require any chemical modification on the probes or the analytes and is based on conformational modifications of the conjugated backbone of a cationic poly(3-alkoxy-4-methylthiophene) when mixed with ss-DNA and a target protein. This procedure could provide an inexpensive means for the rapid detection and identification of various target proteins (including pathogenic proteins) as well as interesting tools for high-throughput screening for drug discovery. Extension toward multiparametric solid-state analyses are also possible and will be tested in the near future.

Experimental Section

UV−Vis Measurements. All UV−vis absorption spectra were taken on a Hewlett-Packard (model 8452A) spectrophotometer.

(a) **Detection of Cations.** In a quartz cuvette with an optical path length of 1.0 cm, 4 μL [2.9 × 10^{-9} mol (based on negative charges)] of 15-mer X1 was added to 100 μL of the aqueous solution of a given alkali metal cation (10 mM) (chloride salts), followed by 4 μL [2.9 × 10^{-9} mol (based on positive charge)] of solution of the cationic polymer **1**. All UV−vis absorption spectra were recorded at room temperature.

(b) **Detection of Human α-Thrombin.** In a 1.0 cm path length UV quartz cell, 1.9 × 10^{-10} mol of human α-thrombin (purchased from Haematologic Technologies Inc., and the initial concentrated solution of thrombin was diluted with sterilized water to obtain the appropriate concentration) and 2.9 × 10^{-9} mol (based on monomeric negative charge or 1.9 × 10^{-10} mol of 15-mer) of ss-DNA thrombin aptamer X1 were mixed in 100 μL of pure water at 25 or 5 °C (it is worth noting that the complex polymer **1**/ssDNA thrombin aptamer/thrombin is more stable at 5 °C than at 25 °C), followed by addition of 2.9 × 10^{-9} mol (based on charge repeat unit) of polymer **1** to form a complex (1/1/1). Two control experiments were carried out with a nonspecific sequence X2 and BSA (bovine serum albumin obtained from Sigma), under identical conditions.

(25) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537.
(26) Chen, L.; McBranch, D. W.; Wang, H. L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U. S.A.* **1999**, *96*, 12287.
(27) Fan, C.; Plaxco, K. W.; Heeger, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 5642.
(28) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 896.
(29) Fan, C.; Hirasa, T.; Plaxco, K. W.; Heeger, A. J. *Langmuir* **2003**, *19*, 3554.
(30) Nilsson, K. P. R.; Andersson, M. R.; Inganäs, O. *J. Phys. Condens. Matter.* **2002**, *14*, 10011.
(31) Nilsson, K. P. R.; Inganäs, O. *Nat. Mater.* **2003**, *2*, 419.
(32) Nilsson, K. P. R.; Rydberg, J.; Baltzer, L.; Inganäs, O. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10170.
(33) Wang, S.; Bazan, G. C. *Adv. Mater.* **2003**, *15*, 1425.
(34) Wilson, J. N.; Wang, Y. Q.; Lavigne, J. J.; Bunz, U. H. F. *Chem. Commun.* **2003**, 14, 1626.

Fluorescence Measurements. All fluorescence spectra were recorded on a Carry Eclipse (Varian Inc.) spectrofluorometer. The excitation was made at 420 nm. In a 3-mm path length fluorescence cell, 3.8×10^{-10} mol of human thrombin and 5.7×10^{-9} mol (based on monomeric negative charge or 3.8×10^{-10} mol of 15-mer) of ss-DNA thrombin aptamer X1 were mixed in 200 μL of pure water, followed by addition of 5.7×10^{-9} mol (based on charge repeat unit) of polymer **1**. The fluorescence spectrum of all mixtures were recorded at 5 °C. For the lower concentration of human α-thrombin, the excitation used was at 420 nm and the fluorescence emission intensity was measured at 584 nm (without recording the entire emission spectrum).

Acknowledgment. Financial support of this research by the Natural Sciences and Engineering Research Council of Canada, the Canada Research Chair Program, and Infectio Diagnostic Inc. is gratefully acknowledged.

JA037289F