Interactions between a Zwitterionic Polythiophene Derivative and Oligonucleotides As Resolved by Fluorescence Resonance Energy Transfer

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The interactions between a zwitterionic polythiophene derivative, POWT, and DNA oligonucleotides in solution have been studied by FRET (fluorescence resonance energy transfer). When POWT and ssDNA are bound alone in a complex, the distance between them is at its smallest. The distance increases when adding complementary DNA, but POWT is still mainly bound to the first DNA strand. We find that two POWT chains bind to one DNA strand, and the two POWT chains seem held together in pairs, unable to separate, as they can only bind to and quench half their own amount of labeled DNA. This POWT−POWT complex appears to dissociate at lower concentrations. ssDNA attached to POWT in a complex can also be substituted by other ssDNA in solution; this occurs to 50% when the free DNA is present in 10-fold concentration compared to the ssDNA bound to POWT. Titration studies at different concentrations show positive cooperativity in the binding of POWT and ssDNA into a complex. The hybridization of complementary DNA to the same complex involves no cooperativity. These observations indicate interesting possibilities for the use of POWT as a DNA sensor.

Introduction

One of the tools of biotechnology is biosensors. The use of conjugated polymers as biosensor devices is a growing research field, and the detection of small quantities of biomolecules is of great interest. Areas in which detection of DNA is of interest are for example forensic science, medical diagnostics, and the study of mutations.[1,2] Indeed, the genomic revolution creates a great need for cheap methods for DNA detection and decoding.

DNA is also an object of present day nanotechnology, being a template with a uniquely high aspect ratio between width and length.[3] The possibility to use DNA chains to label nano-objects is exploited,[4,5] and developments toward the use of such DNA-labeled nano-objects for assembly is well on the way. Therefore, DNA may well be a molecule of choice for the assembly of nanostructured materials or for building of systems from heterogeneous objects of nanometer dimension. Therefore, the limits to assembly and to positioning of small objects with the help of DNA macromolecules are topics of great interest.

One approach to DNA detection is to use the special properties of conjugated polymers, which can respond to external stimuli, such as biomolecules, with a change in fluorescence spectra.[6−11] One of the advantages with this type of sensor is the collective system response given by the series of chromophores building the polymer chain.[12] There are reports describing polythiophenes with DNA-recognition properties in the literature.[9−11,13,14] The molecule investigated in this paper is a polythiophene derivative denoted POWT, poly(3-[(s)-5-amino-5-carboxyl-3-oxapentyl]-2,5-thiophenylene hydrochloride) (Figure 1). The interactions between

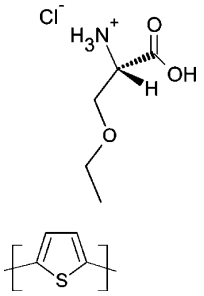

Figure 1. Monomer repeat unit of POWT.

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(1) Liu, B.; Gaylord, B. S.; Wang, S.; Bazan, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 6705−6714.
(2) Balakin, K. V.; Korshun, V. A.; Mikhalev, I. I.; Maleev, G. V.; Malakhov, A. D.; Prokhorenko, I. A.; Berlin Yu, A. *Biosens. Bioelectron.* **1998**, *13*, 771−778.
(3) Maubach, G.; Fritzsche, W. *Nano Lett.* **2004**, *4*, 607−611.
(4) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. *Nature* **1996**, *382*, 609−611.
(5) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882−1884.
(6) Wang, D.; Gong, X.; Heeger, P. S.; Rininsland, F.; Bazan, G. C.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 49−53.
(7) Heeger, P. S.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12219−12221.
(8) Pande, R.; Kamtekar, S.; Ayyagari, M. S.; Kamath, M.; Marx, K. A.; Kumar, J.; Tripathy, S. K.; Kaplan, D. L. *Bioconjugate Chem.* **1996**, *7*, 159−164.
(9) Ewbank, P. C.; Nuding, G.; Suenaga, H.; McCullough, R. D.; Shinkai, S. *Tetrahedron Lett.* **2001**, *42*, 155−157.
(10) Levesque, I.; Leclerc, M. *Synth. Met.* **1997**, *84*, 203−204.
(11) Leclerc, M.; Dufresne, G.; Blondin, P.; Bouchard, J.; Belletete, M.; Durocher, G. *Synth. Met.* **2001**, *119*, 45−48.
(12) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 7017−7018.
(13) Korri-Youssoufi, H.; Garnier, F.; Srivastava, P.; Godillot, P.; Yassar, A. *J. Am. Chem. Soc.* **1997**, *119*, 7388−7389.
(14) Dore, K.; Dubus, S.; Ho, H. A.; Levesque, I.; Brunette, M.; Corbeil, G.; Boissinot, M.; Boivin, G.; Bergeron, M. G.; Boudreau, D.; Leclerc, M. *J. Am. Chem. Soc.* **2004**, *126*, 4240−4244.

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POWT and biomolecules, as well as the response of POWT to other external stimuli, have been studied previously.[15−20]

POWT shows different fluorescence spectra depending on whether it is free in solution, bound to single-stranded DNA (ssDNA), or bound to double-stranded DNA (dsDNA).[16] The cause of the change in emission is thought to be a conformation change in the polymer backbone, from a planar to a nonplanar conformation or vice versa. As the transition takes place, a change in the π-electron overlap occurs, altering the effective conjugation length of the polymer. Thus, the planar conformation corresponds to a high conjugation length of the backbone, while the nonplanar conformation decreases the conjugation length.[11,15] In addition to the change in conjugation length, a planar conformation also increases the possibility of interactions and aggregation between chains, which alters the optical response through interchain transitions.[21] The emission is characterized by an intrachain process at around 540 nm and an interchain process at 670 nm.[16,19]

When binding ssDNA, the positive amino groups of the POWT side chain may interact electrostatically with the negatively charged phosphate backbone of ssDNA. This will give a more planar conformation of the polymer, which can be seen as a decrease of intensity and a shift of the fluorescence to longer wavelengths (red shift), around 590 nm. Due to the planar conformation, interactions with nearby chains, electrostatically or by hydrogen bonds, can lead to aggregation,[16] which is seen as a shoulder in the emission profile at around 670 nm. Other groups have shown that the interaction between the negative backbone of DNA and positively charged groups of a polymer are primarily due to cooperative electrostatic forces, but also hydrophobic interactions between DNA bases and aromatic polymer units contribute.[1,22] It has also been found that aggregated states have weaker emission, which is usually attributed to enhanced mobility of excitons and, thus, increased quenching.[23,24]

When a complementary DNA strand is added to a complex of POWT and ssDNA, dsDNA is formed. Since the dsDNA complex is larger it separates the polymer chains and aggregation disappears, as does the emission shoulder at 670 nm. As the dsDNA forms a helical structure the backbone of POWT is distorted. Thus, the effective conjugation length will decrease, giving a blue shift to around 580 nm, and the possibility of an excited state to diffuse and find a quenching center is lowered, which increases the fluorescence intensity. The photoluminescence emission of POWT is, thus, the result of a subtle balance between intrachain processes, giving a signal at lower wavelengths, and interchain processes, at higher wavelengths, so a detection parameter taking advantage of both is preferable. Using the ratio of the intensities at 540/670 nm (intrachain and interchain, respectively) gives a reproducible measurement, as an internal standard is automatically obtained.[16]

This model needs testing to determine the mechanisms behind the interactions between POWT and DNA. This paper reports the results of studies of the interactions between POWT and DNA using absorbance and fluorescence measurements. The interactions between chromophore-labeled DNA probes and POWT have been studied by means of fluorescence resonant energy transfer (FRET). Unlabeled DNA probes have been used in titration experiments to gain knowledge about the interactions between POWT and DNA. From these data we evaluate models of both mechanisms and geometry in POWT/DNA interactions.

Experimental Section

Materials. The synthesis of POWT was reported elsewhere.[18] A stock solution of 0.5 mg/mL was prepared in deionized water (Milli-Q), which corresponds to a concentration of 2.34 mM on a monomer basis. POWT has a dispersion of chains with lengths primarily between 13 and 19 monomers, as determined from matrix-assisted laser desorption ionization time-of-flight spectroscopy.[17]

Four sets of oligonucleotides were used: the 19-mer P1, 5′-CCG CCA GCG CAG GAA GCT G-3′; the complementary P2, 5′-CAG CTT CCT GCG CTG GCG G-3′; P2 labeled with the dye AMCA at the 5′-end, referred to as P2(AMCA) to avoid confusion considering complementarity; and P3, 5′-CAT GAT TGA ACC ATC CAC CA-3′, noncomplementary to the others and used as the negative control. The stock solutions of DNA were of the concentration 100 nmol/mL (100 μM). P1 and P2 used in the FRET experiments and P3 were purchased from SGSDNA, Köping, Sweden. P1 and P2 used in titration experiments were purchased from MWG Biotech AG, Ebersberg, Germany. P2(AMCA) was purchased from Thermo Electron GmbH, Ulm, Germany.

A buffer solution of 20 mM Na phosphate + 50 mM NaCl (pH 7.4) was prepared and used in the FRET experiments. A Tris-HCl buffer, 20 mM pH 7.4, was used in the titration experiments. Deionized water of Milli-Q quality was used for preparation.

Technical Equipment. Absorbance measurements were done in a Perkin-Elmer Lambda 9 UV/vis/NIR spectrophotometer. FRET measurements were done with an ISA Jobin-Yvon spex Fluoro-Max-2 spectrometer (slit width 2−6 nm). Titration experiments were done in a BMG Fluostar Galaxy microplate reader [excitation 355 nm (38 nm full width at half-maximum) or 390 nm (20.5 nm), emission 460 nm (24 nm), 520 nm (33 nm), and 590 nm (11.5 nm)]. In all measurements blank subtraction was done.

General Notation. The experimental procedure and the complexes formed between POWT and DNA in the following experiments are denoted as follows. The first step, when an oligonucleotide is reacting with POWT and forms a POWT−ssDNA complex, is called the complexation step. The second step, when complementary ssDNA is added, is called the hybridization step. The complexes are denoted as, for example, POWT−P1 + P2, which

(15) Nilsson, K. P. R.; Andersson, M. R.; Inganas, O. *J. Phys.: Condens. Matter* **2002**, *14*, 10011−10020.
(16) Nilsson, K. P.; Inganas, O. *Nat. Mater.* **2003**, *2*, 419−424.
(17) Nilsson, K. P. R.; Olsson, J. D. M.; Konradsson, P.; Inganas, O. *Macromolecules* **2004**, *37*, 6316−6321.
(18) Andersson, M.; Ekeblad, P. O.; Hjertberg, T.; Wennerström, O. *Polym. Commun.* **1991**, *32*, 546−548.
(19) Berggren, M.; Bergman, P.; Fagerstrom, J.; Inganas, O.; Andersson, M.; Weman, H.; Granstrom, M.; Stafstrom, S.; Wennerstrom, O.; Hjertberg, T. *Chem. Phys. Lett.* **1999**, *304*, 84−90.
(20) Björk, P.; Persson, N.-K.; Nilsson, K. P. R.; Åsberg, P.; Inganäs, O. *Biosens. Bioelectron.* **2004**, *20*, 1764−1771.j.bios.2004.07.001.
(21) Theander, M. *Photoluminescence in conjugated polymers*; Department of Physics and measurement technology, Linköping University: Linköping, Sweden, 2000.
(22) Xu, Q. H.; Gaylord, B. S.; Wang, S.; Bazan, G. C.; Moses, D.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11634−11639.
(23) Langeveld-Voss, B. M. W.; Janssen, R. A. J.; Meijer, E. W. *J. Mol. Struct.* **2000**, *521*, 285−301.
(24) Cornil, J.; Heeger, A. J.; Bredas, J. L. *Chem. Phys. Lett.* **1997**, *272*, 463−470.

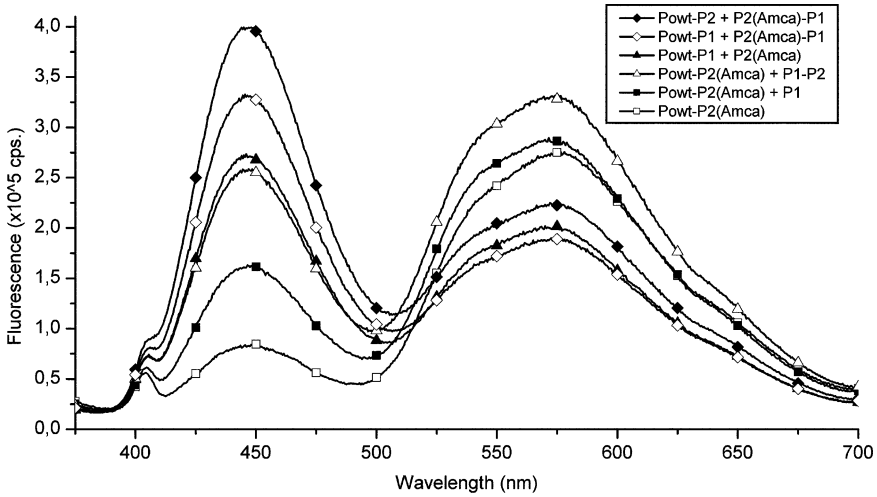

Figure 2. Fluorescence spectra of P2(AMCA) (left peaks) in the presence of POWT (right peaks).

mean that POWT and P1 first are allowed to bind in the complexation step, after which P2 is added in the hybridization step.

General Experimental Procedures. All experiments follow the same procedure. Three different types of complexes are studied: POWT bound only to ssDNA in a POWT−ssDNA complex (for example, POWT−P2), the same complex with the addition of complementary or noncomplementary ssDNA in the hybridization step (POWT−P2 + P1), and that with the addition of pre-hybridized dsDNA (POWT−P2 + P1−P2). The proper amounts of POWT and ssDNA are mixed to form the POWT−ssDNA complex. Milli-Q water is added in an amount to make the final volume of POWT, DNA, and Milli-Q water equal to 100 mL. The mixture is incubated for 15 min. If dsDNA is to be added in the hybridization step, the proper amounts of DNA are mixed in buffer and left to hybridize for 15 min. After incubation, the complementary ssDNA (or noncomplementary ssDNA or dsDNA) is mixed with the POWT−ssDNA complex. Buffer is added to the final volume (1000 mL in the cuvette, 350 mL in the well). The sample is incubated for 15 min before the measurement is performed.

Measurements are done for different concentrations of POWT with the addition of different amounts of DNA, also expressed as ratios in relation to POWT. The volume of POWT solution used is 10 μL. This corresponds to a monomer concentration of 23.4 μM in the cuvettes and 66.8 μM in the microplate wells if the stock solution is used. At the POWT−DNA ratio of 1:1 (one POWT monomer to one DNA base) this corresponds to the DNA concentrations of 1.37 μM (cuvettes) and 3.93 μM (microplate) on a molecule basis. For diluted samples the concentration changes correspondingly. POWT−DNA ratios relate the number of monomers of POWT with the number of DNA bases, or rather DNA phosphate groups, as binding is believed to take place between the positive charges of the polymer side chain and the negative phosphate groups of the DNA backbone.

FRET Experiments. FRET efficiencies and distances were determined for the complexes POWT−P2(AMCA), POWT−P2(AMCA) + P1, POWT−P1 + P2(AMCA), POWT−P2(AMCA) + P1−P2, POWT−P1 + P1−P2(AMCA), and POWT−P2 + P1−P2(AMCA). In all experiments the POWT−DNA ratio was 1:0.5, and stock solutions were used. For the complex POWT−P2(AMCA) the measurements were repeated for the ratio 1:0.5 and also done for the ratio 1:1.

Substitution Experiment. Samples of POWT−P2(AMCA) were titrated with P2 and measured in the microplate reader. The POWT−P2(AMCA) ratio was 3:1 to ensure that all AMCA molecules were bound and, thus, quenched. The concentration of POWT−P2(AMCA) was held constant, and P2 was titrated at different ratios from 0.3 to 300. The samples were diluted 10 and 100 times. The experiment was designed to find out whether DNA in solution could substitute DNA bound to POWT. Every sample was measured in three separate wells.

Titration Experiments. To investigate the interaction between POWT and DNA, fluorescence titration experiments were done in the microplate reader for POWT−P2 (POWT concentration held constant and P2 titrated) and POWT−P2 (1:1) + P1 [POWT−P2 (1:1) concentration held constant and P1 titrated]. The samples were diluted from 3 up to 300 times. Control experiments of POWT−P2 + P3 diluted 10 times were also performed. Every sample was measured in at least five separate wells. The signal was measured as the change of the ratios of the intensities at 520 and 590 nm, I_{520}/I_{590}. The data obtained were analyzed using nonlinear regression in OriginPro 7.

Results and Discussion

FRET Experiments. FRET is a dipole−dipole coupling process in which energy is transferred from an excited donor chromophore to an acceptor chromophore.[25] The emission of the donor (AMCA) in the absence (F_0; Figure 3) or presence (F) of the acceptor (POWT; Figure 2) is used for calculating the transfer efficiency, E, given by[26]

$$E = 1 - (F/F_0) \tag{1}$$

The overlap integral, J, correlating donor emission and acceptor absorption, is[26]

$$J = \int F_D(\lambda)\, \epsilon(\lambda) \lambda^4\, d\lambda \tag{2}$$

where F_D is the peak-normalized fluorescence spectrum of the donor, ϵ is the absorption spectra of the acceptor (Figure 3), and λ is the wavelength. The Förster distance, R_0, at which the transfer efficiency is 50% can be calculated by[26]

$$R_0^6 = 8.785 \times 10^{-5} k^2 Q_D J / n^4 \tag{3}$$

Finally, the actual (or at least relative) distance between the molecules, R, can be obtained by[26]

$$R = R_0 (1/E - 1)^{1/6} \tag{4}$$

Using the experimental data, the Förster transfer efficiencies and the Förster distances were calculated (Table 1). The

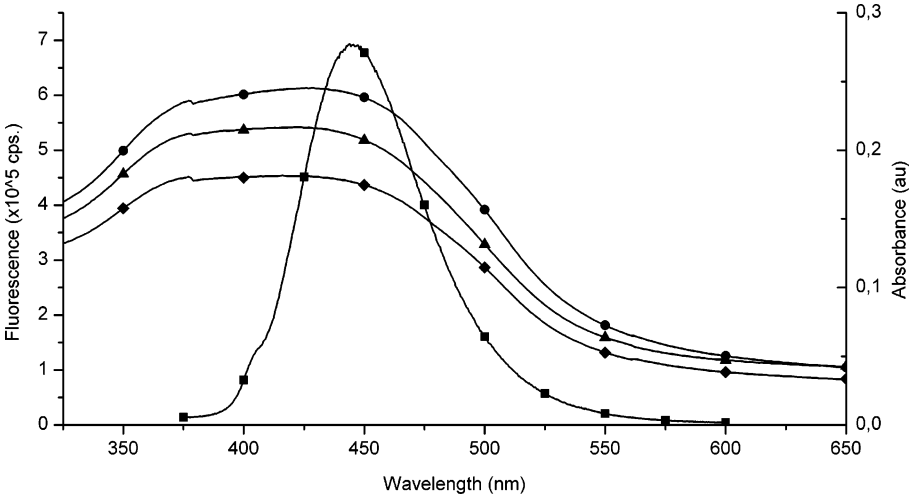

Figure 3. Fluorescence spectra of P2(AMCA) (squares) and absorbance spectra of POWT−P1 (circles), POWT−P1 + P2 (triangles), and POWT−P1 + P1−P2 (diamonds).

Table 1. Förster Distances (R_0), Efficiencies, and Distances (R) between Donor [P2(AMCA)] and Acceptor (POWT) in Different POWT−DNA Complexes

complex (ratio 1:0.5)	R_0 (Å)	efficiency (%)	R (Å)
POWT−P2(AMCA)	26.8	85.7	19.9
POWT−P2(AMCA) + P1	26.2	74.6	21.9
POWT−P2(AMCA) + P1−P2	25.4	60.8	23.6
POWT−P1 + P2(AMCA)	26.2	59.4	24.6
POWT−P1 + P1−P2(AMCA)	25.4	50.8	25.3
POWT−P2 + P1−P2(AMCA)	25.4	40.9	27.0
repeated measurements (ratio)			
POWT−P2(AMCA) (1:0.5)	26.8	85.9	19.8
POWT−P2(AMCA) (1:1)	26.8	63.7	24.4

refractive index (n) of the medium was set to 1.4,[26] and the quantum yield for AMCA (Q_D) is given in the literature as 0.49.[27] The dipole orientation factor (k^2) is assumed to be $^2/_3$.[28]

From these results some interesting conclusions can be made. First, the Förster distances (R_0) are all close to 26 Å, which is a normal value.[26,29] The efficiencies also take on values in a range where Förster theory is valid (below 10 and above 98% the errors are larger).[30] The range of the distances, between 19 and 27 Å, is also reasonable compared to those in the literature.[22] However, there can be deviations from the Förster theory when studying polymers.[31,32] The obtained Förster distances in a complex system are not one unique, but rather an average, of the existing donor−acceptor distances.[30,33] As a result of the strong dependence of transfer efficiency on the intermolecular distance, however, the closest chromophore will probably be of most influence.[34] Also, the nature of the excited states of the polymer cannot be treated as static, tightly bound dipoles. Instead they are relatively weakly bound, and their wave functions are delocalized over possibly a few nanometers of distance. The assumption of k^2 as $^2/_3$ introduces some uncertainty in the obtained distances. However, Stryer[28] shows that the uncertainty is rather small, and Xu and co-workers[22] estimate the error due to the assumption to be less than 10%. This uncertainty should not affect the order of distances between the different complexes if the relative orientation of donor and acceptor is the same in all complexes. This calls for some care when analyzing FRET experiments, and the obtained distances are better seen as relative than actual.

The graph of the POWT−P2(AMCA) complexes (Figure 2) has, at least partially, a crossover between donor and acceptor emission, which is preferable. A low donor signal indicates high transfer efficiency, which should give a high acceptor signal. The crossover is not perfect, which could be attributed to the features of POWT. POWT can hardly be considered as a perfect acceptor for the reasons stated above. However, AMCA is considered a good donor.[27] Because it is the donor signal and not the acceptor signal that is used to calculate transfer efficiency, the results of the POWT−P2(AMCA) complexes can be considered valid.

The distances obtained for the POWT−P2(AMCA) complexes are shown in Table 1. The smallest distances are obtained when P2(AMCA) is attached directly to POWT in the complexation step. It is smallest when no additional DNA is added [19.9 Å, POWT−P2(AMCA)] and largest when dsDNA is added [23.6 Å, POWT−P2(AMCA) + P1−P2]. Thus, adding complementary DNA to a POWT−ssDNA complex rearranges the molecules. When P2(AMCA) is added in the hybridization step, the distance is smallest when it is added as complementary ssDNA [24.6 Å, POWT−P1 + P2(AMCA)] and largest when added as dsDNA and P2-(AMCA) is not complementary to the POWT−ssDNA complex [27.0 Å, POWT−P2 + P2(AMCA)−P1]. The value of 19.9 Å for the POWT−P2(AMCA) complex is in good agreement with studies recently performed by Xu and co-workers.[22] They found, depending on experimental setup, one distance of 21 Å in which the interaction between a polymer and DNA was both hydrophobic and electrostatic

(25) Förster, T. *Ann. Phys. (Leipzig)* **1948**, *2*, 55−75.
(26) Wu, P.; Brand, L. *Anal. Biochem.* **1994**, *218*, 1−13.
(27) Malicka, J.; Gryczynski, I.; Fang, J.; Kusba, J.; Lakowicz, J. R. *Anal. Biochem* **2003**, *315*, 160−169.
(28) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819−846.
(29) Dogariu, A.; Gupta, R.; Heeger, A. J.; Wang, H. *Synth. Met.* **1999**, *100*, 95−100.
(30) Dietrich, A.; Buschmann, V.; Muller, C.; Sauer, M. *J. Biotechnol.* **2002**, *82*, 211−231.
(31) Burlatsky, S. F.; Oshanin, G. S.; Mogutov, A. V. *Phys. Rev. Lett.* **1990**, *65*, 3205−3208.
(32) Roy, A. K.; Blumen, A. *J. Chem. Phys.* **1989**, *91*, 4353−4359.
(33) Loura, L. M. S.; Almeida, R. F. M.; Prieto, M. J. *J. Fluoresc.* **2001**, *11*, 197−209.
(34) Andrew, P.; Barnes, W. L. *Science* **2000**, *290*, 785−788.

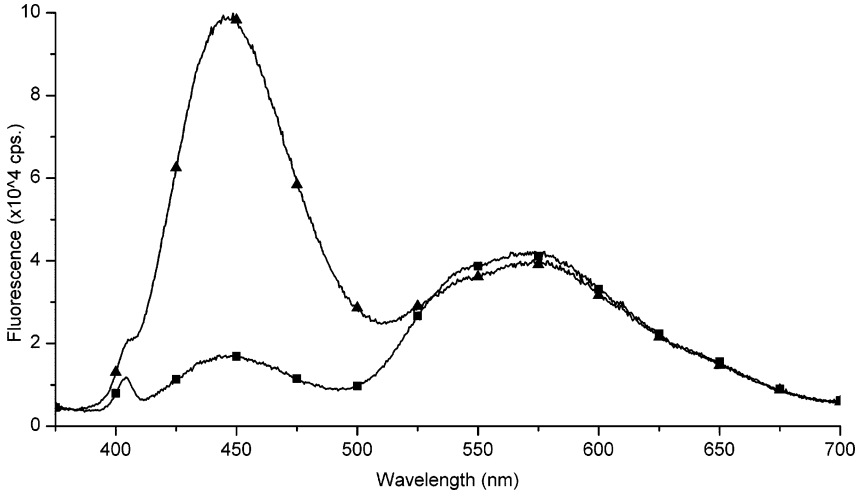

Figure 4. Fluorescence spectra of POWT−P2(AMCA) with ratios of 1:0.5 (squares) and 1:1 (triangles).

and one of 30 Å when only electrostatic interactions were present. The distance of 19.9 Å found in this paper is probably due to both hydrophobic and electrostatic forces. When adding more DNA the repulsive forces between the negative phosphate groups in the DNA backbone will increase, separating the molecules.

When the distances of POWT−P2(AMCA) + P1 and POWT−P1 + P2(AMCA) (21.9 and 24.6 Å, respectively) are compared, it can be seen that POWT does not bind in the same way to the two DNA strands in the formed dsDNA. Thus, even if a normal dsDNA helix is formed when complementary DNA is added,[16] POWT is still bound stronger to the first DNA strand. Noteworthy are also the small differences in distance between the three cases where dsDNA is added in the hybridization step (24.6, 25.3, and 27.0 Å). P2(AMCA) is obviously connected to POWT in all cases, which means that a complex of three DNA strands together with POWT is formed. No breaking up of the initial POWT−ssDNA complex or of the dsDNA takes place. This can be of importance in sensor applications, as samples might not need denaturation before detection. Bear in mind, however, that it is difficult to determine the errors of the obtained distances. A difference of 2 Å is not much, and the significance is quite low. The order of the distances of the complexes could be different in reality, although a visual inspection of Figure 2 reveals large differences in intensity and the order of distances is quite logical. In all, the results should be treated with some caution. The purpose of the study was to investigate distances between binding oligonucleotides and polymer. Interactions with noncomplementary DNA were studied in hybridization step binding experiments (later, Figure 8) and substitution experiments (later, Figure 6).

The measurements for the complex POWT−P2(AMCA) were repeated for the same ratio as used above (1:0.5) and also done for the ratio 1:1, that is, equivalent amounts of POWT and DNA (Figures 4 and 5). For the ratio of 1:0.5 the result is identical to the one obtained in the previous measurement, with a distance of 19.8 Å (Table 1). However, for the ratio of 1:1, the results are quite different. The calculated distance increases to 24.4 Å, and the emission of the donor, AMCA, is much larger for the ratio 1:1, while

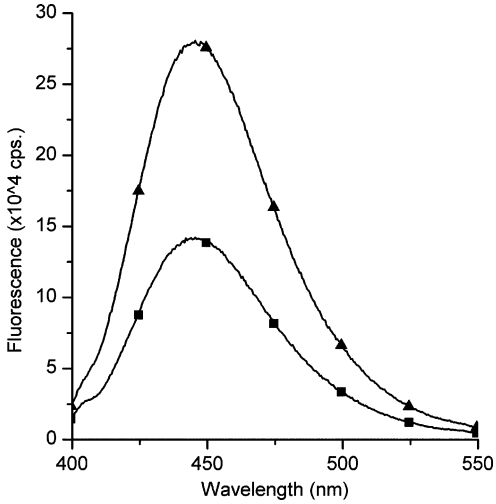

Figure 5. Fluorescence spectra of P2(AMCA) in the absence of POWT, with amounts of P2(AMCA) equivalent to ratios of 1:0.5 (squares) and 1:1 (triangles).

the emission of the acceptor, POWT, is almost the same (Figure 4). Thus, P2(AMCA) is not bound to POWT, and it seems as though all POWT chains have bound DNA already at the ratio 1:0.5. Adding more DNA does not affect the POWT chains. In fact, the result indicates that the POWT chains are held together in pairs, unable to separate, because they can only bind to and quench half their own amount of DNA. This gives valuable information about the conformation and geometry of POWT. Here it is appropriate to raise the question whether even the ratio 1:0.5 is too high, which would result in the presence of P2(AMCA) not bound to POWT and, thus, to excessive calculated distances. However, considering the high transfer efficiency (86%), almost all P2(AMCA) is probably bound to POWT. Higher transfer efficiency would shorten the distances obtained with a few nanometers. Further studies and molecular modeling need to be made to determine the actual interaction.

Substitution Experiments. POWT−P2(AMCA) at the ratio of 3:1 was titrated with P2 to see whether any substitution occurred, which would be noticed as an increase in the P2(AMCA) emission due to decreased quenching. It is evident that this happens; at approximately 10 times greater amount of P2 in solution, compared to P2(AMCA) bound

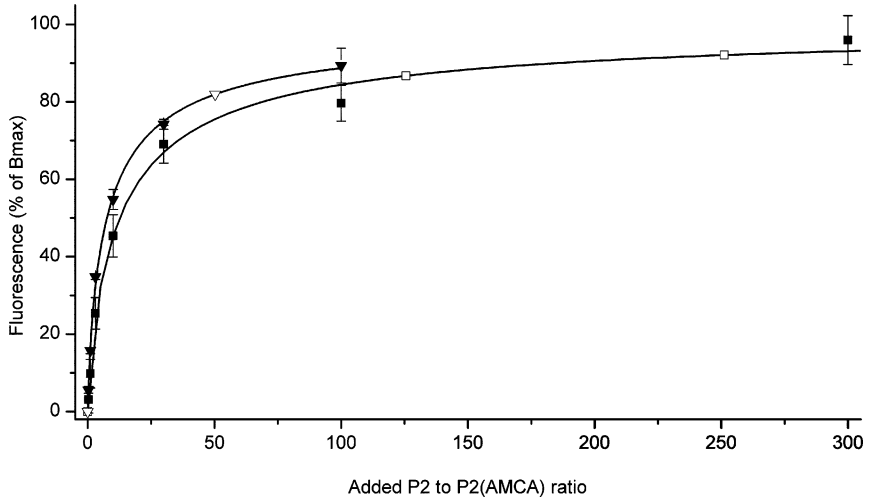

Figure 6. Fluorescence change of POWT−P2(AMCA) (3:1) at 460 nm, titrated with P2. Samples diluted 10 times (triangles) and 100 times (squares). Filled symbols show original data; open symbols show fitted values. Data are fitted with the cooperative binding model (Hill) with $K_{1/2} = 7.6$ and 12.6, respectively. Error bars represent standard deviation.

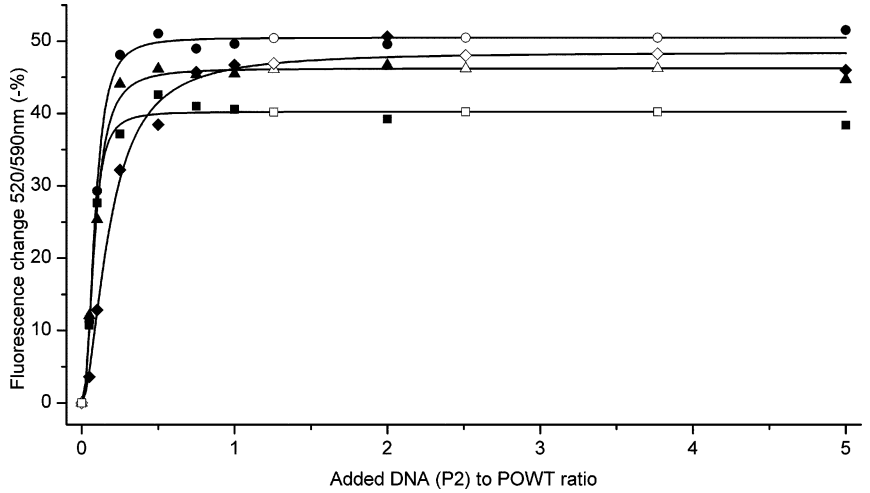

Figure 7. Titration of P2 to POWT diluted 3 (squares), 10 (circles), 30 (triangles), and 100 (diamonds) times, fitted with the cooperative binding model (Hill). Filled symbols show original data; open symbols show fitted values. Standard deviations (not shown) are as follows: for the sample diluted 3 times, <2.5%; 10 times, <3.5%; 30 times, <6.6%; and 100 times, <16.8%.

to POWT, the signal has reached half-maximum (Figure 6). To get an estimation of the substitution, the data were fitted with the Hill equation (eq 5, next section). The values of $K_{1/2}$ are 7.6 and 12.6 [ratio of added P2 to bound P2(AMCA)] for the sample diluted 10 and 100 times, respectively. This indicates that half of the initially bound P2(AMCA) molecules have been replaced by unlabeled P2 at those ratios.

For sensor applications this is of considerable importance. For a POWT−ssDNA complex of a certain concentration, the concentration of the DNA in the sample to be detected should not be more than approximately 10 times higher. If the sample contains pure noncomplementary DNA, probably no change in signal will occur, as substitution does not change the conformation of POWT (which is still bound to ssDNA, although of a different kind). If it is complementary, however, and at high concentration, it will not merely bind to the POWT−ssDNA complex inducing a detectable signal but also eventually replace the ssDNA initially bound to POWT and, hence, give no change in signal at all. If a mixed sample, with both complementary and noncomplementary DNA, is to be measured, substitution can take place and lower the change in signal. However, a series of POWT−ssDNA complexes at different concentrations could determine the sample DNA concentration, at least within certain limits, which would give not only a qualitative but also a quantitative measurement.

Complexation Step Binding. The complex POWT−P2 was studied with fluorescence titration experiments (titration of P2 to POWT) at different concentrations of POWT (Figure 7). It was found that a simple one-site binding model did not fit the data to a high degree (fit not shown), which is not surprising because the mechanism is probably more complex (a conformational change is involved). Instead the data showed signs of cooperativity, something that has been reported previously for polymer and DNA systems involving conformational changes and electrostatic and hydrophobic interactions.[35−38] Therefore, the data were fitted with a

(35) Jezewska, M. J.; Rajendran, S.; Bujalowski, W. *J. Mol. Biol.* **1998**, *284*, 1113−1131.
(36) Inobe, T.; Makio, T.; Takasu-Ishikawa, E.; Terada, T. P.; Kuwajima, K. *Biochim. Biophys. Acta* **2001**, *1545*, 160−173.
(37) Isogai, N.; Narita, T.; Chen, L.; Hirata, M.; Gong, J. P.; Osada, Y. *Colloids Surf., A* **1999**, *147*, 189−201.
(38) Petrov, A. I.; Khalil, D. N.; Kazaryan, R. L.; Savintsev, I. V.; Sukhorukov, B. I. *Bioelectrochemistry* **2002**, *58*, 75−85.

Table 2. Results of Complexation Step Titration Experiments of POWT (Constant Concentration) + P2 (Titrated), for Samples Diluted from 3 to 300 Times

times diluted	[POWT][a] (μM)	n_H[b]	$K_{1/2}$[c] (ratio)	$K_{1/2}$[d] ([P2]) (nM)
3	22.3	2.54	0.074	97
10	6.7	2.46	0.086	34
30	2.23	2.19	0.086	11
100	0.67	1.76	0.181	7
300	no fit obtained			

[a] Monomer concentration of POWT in sample. [b] Cooperativity factor. [c] Ratio of number of P2 bases to POWT monomers at $K_{1/2}$. [d] Concentration of P2 molecules at $K_{1/2}$.

cooperative binding model (Hill, eq 5),[36] and although the fits are not perfect, this is a simple model that is easier to interpret than more elaborate ones.

$$Y = B_{max}[X]^{n_H}/(K_{1/2}{}^{n_H} + [X]^{n_H}) \quad (5)$$

B_{max} is the maximum recorded signal, n_H is the degree of cooperativity, where a value higher than 1 indicates positive cooperativity, and $K_{1/2}$ is the concentration at which 50% of the maximum signal is reached. If this level is reached when half of the POWT monomers have bound DNA and all are bound at full signal, $K_{1/2}$ is the same as the equilibrium dissociation constant, K_D. Hence, if half of the POWT molecules have changed their conformation due to the binding of DNA, the signal should then be at 50% of the maximal value, which is reached when all POWT molecules have changed their conformation.

The n_H values are all higher than 1, indicating positive cooperative binding (Table 2). This could be because the binding of DNA to POWT includes a conformational step, as shown previously by surface plasmon resonance.[20] The change in conformation, which can also be seen in the fluorescence spectra, might make it easier for more DNA strands to bind to POWT. At low DNA concentrations, there are probably POWT chains partially bound to DNA, with one end unbound. The change in conformation induced by DNA can affect also those monomers that are still unbound, facilitating the binding of more DNA. Another reason could be the formation of aggregates, due to a planar conformation and hydrophobic interactions. At low DNA concentrations, only a small percentage of the POWT molecules are planarized and, thus, few and small aggregates are formed. With higher DNA concentrations the aggregates increase in size, increasing the intermolecular interactions that are responsible for the shift in fluorescence. It has also been found[1,22] that the interaction between the negative backbone of DNA and positively charged groups of a polymer are primarily due to cooperative electrostatic forces, which could also be the reason. The cooperativity decreases with decreasing concentration (from 2.5 for the sample diluted 3 times to 1.8 for 100 times dilution). This can be due to the formation of smaller aggregates at lower concentrations. This is supported by Xu and co-workers,[22] who found that at low concentrations the hydrophobic interactions decrease, which would give less aggregation.

As seen in the FRET experiments, saturation seems to be reached at the POWT−ssDNA ratio 1:0.5 or below. Again, this indicates that POWT can only bind half its amount of DNA. The explanation is the same as before, that two POWT chains bind together to form a pair, a POWT−POWT complex. This is true for the higher concentration, while the sample diluted 100 times reached saturation at around a 1:1 ratio. The reason for this can be that the POWT−POWT interaction has a binding dissociation constant within this range of concentration, and, thus, the complex separates when the concentration is lowered. It would then require equal amounts of DNA to POWT to reach saturation. This also results in the increasing trend in $K_{1/2}$ values, from a $K_{1/2}$ ratio of 0.07 for the sample diluted three times to 0.18 for 100 times dilution, meaning that it takes more DNA in relation to POWT for a total conformational change at low POWT concentrations. This can also be explained by the decrease of hydrophobic interactions at low concentration, which changes the equilibrium.[22] If this trend continues at even lower concentrations, eventually the point would be reached when lowering the concentration of POWT does not lower the concentration of DNA at $K_{1/2}$. Unfortunately, no fit was obtained for the sample diluted 300 times, and to reach lower concentrations more sensitive equipment is needed. The lowest amount of DNA detected, or rather the lowest $K_{1/2}$ value, for these samples is 7 nM (DNA molecules) for a POWT concentration of 670 nM (monomers). The complexation step curve fitting shows that the actual value of $K_{1/2}$ depends on the concentration of POWT. If $K_{1/2}$ is a good representation of the equilibrium dissociation constant, K_D, describing the binding interacting between POWT and DNA, the actual value lies below what has been possible to measure in this work.

Hybridization Step Binding. The hybridization complex POWT−P2 + P1 was studied with fluorescence titration experiments (titration of P1 to POWT−P2) at different concentrations of POWT−P2 (Figure 8). The POWT−P2 complexes to which P1 was added all had the POWT to P2 ratio of 1:1. The data are fitted with a one-site binding model, given by[39]

$$Y = B_{max}[X]/(K_{1/2} + [X]) \quad (6)$$

where B_{max} is the maximum recorded signal and $K_{1/2}$ is the concentration at which 50% of the maximum signal is reached.

There are two reasons for the 1:1 ratio of the POWT−P2 complex, instead of 1:0.5 where saturation seems to be reached (as found in the previous section). First, for the POWT−ssDNA sample diluted 100 times it was found that the 1:0.5 ratio was not enough to reach saturation. To have the same ratios in all hybridization step measurements, 1:1 was preferable. It was also found that a POWT−P2 ratio of 1:0.5 was most favorable for the formation of aggregates, as was seen by a change in surface tension of the liquid by visual inspection of the wells. These aggregates seemed difficult to break, which gave small conformational changes when adding complementary DNA and, thus, low signals.

A first analysis of POWT−P2 + P1 shows that full conformational change is obtained at the ratio 1:1 or above.

(39) Motulsky, H.; Christopoulos, A. *Fitting models to biological data using linear and nonlinear regression*; GraphPad Software, Inc.: San Diego, CA, 2003.

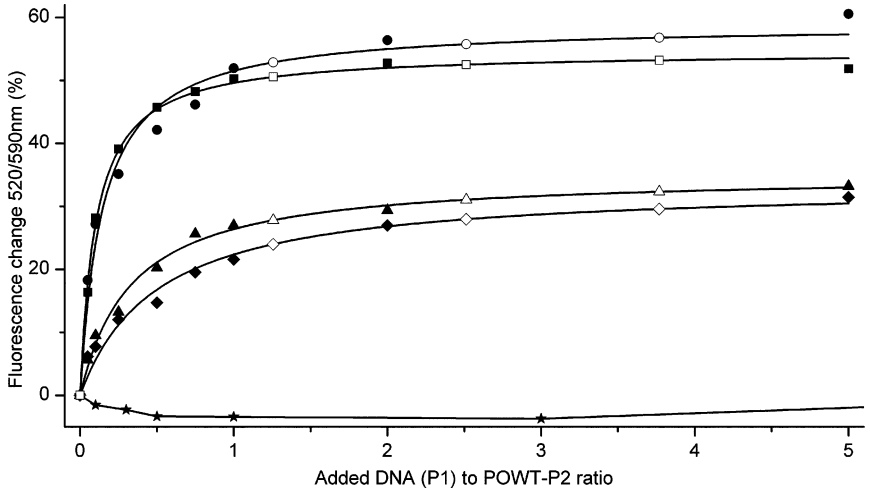

Figure 8. Titration of P1 to POWT−P2 (1:1) diluted 3 (squares), 10 (circles), 30 (triangles), and 100 (diamonds) times, fitted with the one-site binding model, and P3 to POWT−P2 diluted 10 times (stars). Filled symbols show original data; open symbols show fitted values. Standard deviations (not shown) are as follows: for the sample diluted 3 times, <2.1%; 10 times, <4.4%; 30 times, <5.5%; 100 times, <12%; and P3 10 times, <3.5%.

Table 3. Results of Hybridization Step Titration Experiments of POWT−P2 (Ratio 1:1, Constant Concentration) + P1 (Titrated), for Samples Diluted from 3 to 300 Times

times diluted	[POWT][a] (μM)	$K_{1/2}$[b] (ratio)	$K_{1/2}$[c] ([P1]) (nM)
3	22.3	0.10	131
10	6.7	0.14	55
30	2.23	0.33	43
100	0.67	0.50	20
300	no fit obtained		

[a] Monomer concentration of POWT in sample. [b] Ratio of number of P1 bases to POWT monomers at $K_{1/2}$. [c] Concentration of P1 molecules at $K_{1/2}$.

Because the one-site binding model is used, no cooperativity is present. There is a conformational change when complementary ssDNA binds the POWT−ssDNA complex, but it does not seem to affect the binding of the hybridization DNA. The results are what could be expected, as there is only one perfect binding site between the complementary DNA strands and saturation is reached close to the ratio 1:1, when there are equal amounts of the two strands. Titration of the noncomplementary P3 gives no signal at all.

The relative trend of $K_{1/2}$ ratios in the dilution series is increasing, from 0.10 to 0.50, suggesting that eventually, at low enough POWT concentrations, a limit is reached, at which lowering the POWT concentration further does not change the actual DNA concentration at $K_{1/2}$ (Table 3). Again, this could be explained by a change of the interactions from both hydrophobic and electrostatic to only electrostatic. The lowest $K_{1/2}$ value in this measurement is 20 nM (DNA molecules), for a POWT−P2 concentration of 670 nM (monomers). It is evident that the value of $K_{1/2}$ is very dependent on the POWT concentration. Low concentrations of POWT will detect low concentrations of DNA, provided the measurement device is sensitive enough. Further investigations about the detection possibilities need to be made, and to determine the detection limit, more sensitive measurement methods need to be used.

Conclusion

The interactions between POWT, a zwitterionic polythiophene derivative, and DNA oligonucleotides in solution have been studied. FRET experiments reveal that POWT and ssDNA are at the shortest distance when ssDNA is bound directly to POWT in a POWT−ssDNA complex. Adding complementary DNA increases the distance, but POWT is still mainly bound to the first DNA strand. It was found that two POWT chains bind to one DNA strand, and two POWT chains seem held together in pairs, unable to separate, because they can only bind to and quench half of their own amount of DNA. This POWT−POWT complex appears to dissociate at lower concentrations, where the binding ratio is one POWT chain to one DNA strand. DNA attached to POWT in a POWT−ssDNA complex can also be substituted by other DNA in solution. At a DNA concentration in solution approximately 10 times higher than that of the DNA bound to POWT, 50% has been replaced. The binding of POWT and ssDNA into a complex show positive cooperativity. However, the hybridization of ssDNA to this complex involves no cooperativity. The lowest $K_{1/2}$ measured for the hybridization step binding was 20 nM DNA (on a molecular basis), obtained for a POWT concentration of 670 nM (on a monomer basis). Lower concentrations could not be studied due to equipment limitations; therefore, it is impossible to conclude how low concentrations can be detected. The concentration dependency of detection, with an upper limit set by substitution and a lower limit set by the relative DNA concentration needed to induce a conformational change, should make POWT suitable not only for qualitative detection but also for quantitative analysis of DNA.

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