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Water-Soluble Polyelectrolytes for FRET-based DNA Detection

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Two conjugated polyelectrolytes with same electronic structure with/without molecular spacer (or bumper) are compared in functioning as a donor in fluorescence resonance energy transfer (FRET) to a fluorescein-labeled single-stranded DNA (ssDNA-Fl). Fluorescence quenching of the acceptor (Fl) in the polymer/ssDNA-Fl electrostatic complex is a main concern to improve detection sensitivity. This study can show one important example of structure-property relationships for the FRET-based biosensing assays. Structure modification such as molecular bumper can fine-tune the competition between energy transfer and electron transfer quenching in FRET-based DNA sequence detection.

Keywords: biosensor; conjugated polyelectrolyte; DNA detection; DNA sensor; FRET

INTRODUCTION

Sensitive and selective sequence-specific DNA detection is urgently needed for immediate infection diagnosis [1,2]. Various kinds of biosensors using optical and electrochemical transduction schemes have been proposed and demonstrated [3]. One successful DNA sensing scheme based on the fluorescence resonance energy transfer (FRET) method involves the use of cationic conjugated polyelectrolytes (CCPs)

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and the hybridization between fluorophore-labeled peptide nucleic acids (PNA) and complementary (or non-complementary) DNA [3]. Electrostatic interactions between CCPs and negatively charged DNA can be coordinated to give rise to efficient FRET for sequence-specific DNA detection. This scheme benefits from utilizing the light-harvesting (or antenna-like) properties of CCPs to achieve sensory signal amplification in the presence of suitable energy or electron acceptors.

The overall FRET-based sensing efficiency from these interactions is controlled by the molecular structure of participating species and resulting electrostatic complexes, structural conformation in aqueous medium, the electronic structure of HOMO/LUMO levels of donor and acceptor in combination with several variables such as pH, ionic strength, temperature, etc. [4]. Understanding how these variations come together in complicated biological mixtures is an important requirement for a rational design of the fully optimized FRET-based DNA assays.

In this contribution, we report two water-soluble conjugated polyelectrolytes (**P1i**, **P2i**) designed to modulate the distance D-A in the molecular scale. The main structural difference between two structures is the presence of the anthracenyl substituent on the 9-position of the fluorene comonomer units in **P2**, which is orthogonal to the backbone axis and serves to increase separation between chains in aggregated phases without perturbation in π-conjugation of the backbone (Fig. 1). As detailed below, the two polymers with same electronic conjugation behave differently as an excitation donors to fluorescein-labeled single stranded DNA (ssDNA-Fl). An examination of these

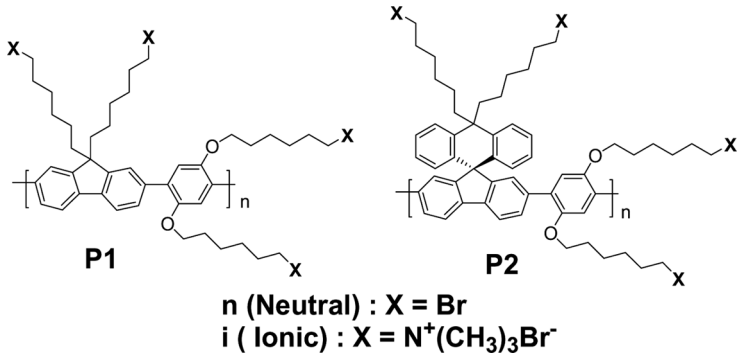

FIGURE 1 Molecular structures of neutral (**P1n**, **P2n**) and ionic polymers (**P1i**, **P2i**).

differences provides insight into the intimate molecular interactions that favor FRET versus energy-wasting PL quenching via electron transfer (eT).

EXPERIMENT

All chemicals were purchased from Aldrich Co., and were used without further purification. ^{1}H- and ^{13}C-NMR spectra were collected on a Varian Unity 400 MHz (or 200 MHz) spectrometer. The UV/vis absorption spectra were recorded on a Shimadzu UV-2401 PC diode array spectrometer. Photoluminescence spectra were obtained on a PTI Quantum Master fluorometer equipped with a Xenon lamp excitation source. Fluorescence quantum yields were measured relative to fluorescein at pH = 11 in water. The oligonucleotides (5′-fluorescein-ATCTT GACTA TGTGG GTGCT-3′) were purchased from Genscript Corp. and DNA concentrations were determined by measuring the absorbance at 260 nm in a 200 μL quartz cuvette.

RESULTS AND DISCUSSION

The neutral precursor polymers (**P1n**, **P2n**) were synthesized by Suzuki coupling using $Pd(PPh_3)_4$ in THF/H_2O (2:1) under reflux over 24 hrs in yields of 40–60%. The degree of polymerization was determined to be $M_n = 19{,}000$ (PDI = 1.42) for **P1n** and $M_n = 29{,}000$ (PDI = 1.69) for **P2n** using GPC (solvent: chloroform), respectively. The water-soluble polyelectrolytes are obtained by treatment of **P1n** and **P2n** with condensed trimethylamine in a THF/water mixture for 24 hrs.

As shown in Table 1, the absorption and photoluminescence (PL) spectroscopy data of **P1i** and **P2i** are almost identical. The same

TABLE 1 Spectroscopy Summary

Polymer	λ_{abs} (nm)	λ_{PL} (nm)	Φ^a	E_{ox} (V)^b	HOMO/LUMO (eV)^c
P1i	362	411	0.43	0.80	−5.6/−2.5
P2i	362	411	0.39	0.83	−5.6/−2.5
DNA-fluorescein	494	513	0.80		−5.8/−3.4

[a]Fluorescence quantum yield relative to fluorescein at pH = 11 in water.

[b]Electrochemical $E_{M+/M}$ is measured by cyclic voltammetry with the neutral polymers (**P1n**,**P2n**) relative to ferrocene in 0.1 M n-Bu_4NPF_6 in acetonitrile.

[c]Approximated by taking into account the ionization potential 4.8 eV for ferrocene and the corresponding bandgap from UV/vis measurements.

electrochemical oxidation potential, $E_{ox} = \sim 0.8\,V$ was measured for both structures relative to ferrocene by CV measurements. Combining of UV/vis and CV measurement confirms both polymers have the identical HOMO and LUMO energy levels. Because of the identical electronic structures, similar FRET behavior to ssDNA-Fl is expected for **P1i** and **P2i**. Measurements were performed in water at pH = 8. Fluorescein was chosen as a FRET acceptor since its absorption is well overlapped with the emission of our polymeric structures. Figure 2 shows the PL spectra of **P2i**/ssDNA-Fl solutions upon excitation of the polymers at 380 nm ([ssDNA-Fl] $= 1.5 \times 10^{-8}$ M, or [base] $= 3 \times 10^{-7}$ M). The emission from Fl is enhanced via FRET with increasing [**P2i**]. However we measured almost no PL from Fl by exciting **P1i** in the case of **P1i**/ssDNA-Fl. Despite the same spectral overlap between donor emission (**P1i** and **P2i**) and acceptor absorption (ssDNA-Fl), different FRET spectra were measured. The difference is believed to be related to the fine structure of electrostatic complexes. Two polymeric systems have same charge density per repeating unit and should have similar electrostatic attraction with ssDNA-Fl, but the D-A distance must be perturbed by the molecular bumper.

According to the equation of long-range Förster-type energy transfer [5]. FRET is strongly dependent on the D-A distance ($k_{FRET} \propto r^{-6}$).

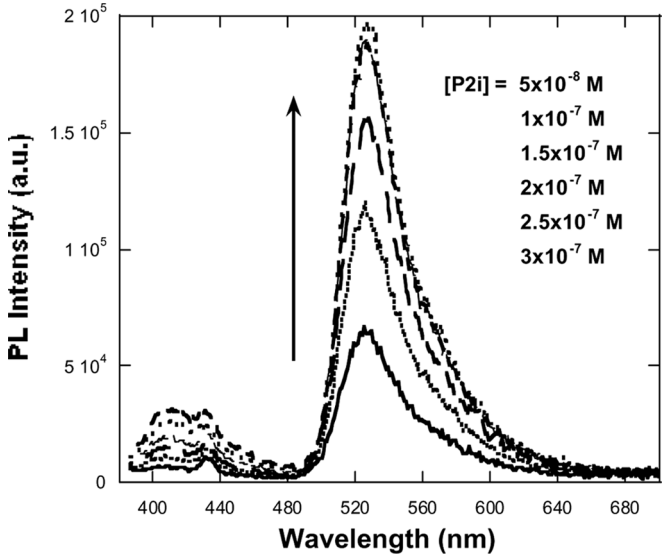

FIGURE 2 PL spectra of **P2i**/DNA-Fl with increasing [**P2i**] in water at pH = 8. $\lambda_{ex} = 380$ nm. [DNA-Fl] $= 1.5 \times 10^{-8}$ M.

The D-A distance in **P1i**/ssDNA-Fl should be shorter than that in **P2i**/ssDNA-Fl with molecular bumper. Both of energy transfer (FRET) and quenching process via electron transfer (eT) are very sensitive to intermolecular distance between donor and acceptor. The electrochemical oxidation potentials by CV measurements show both of **P1i** and **P2i** can be efficient electron donors to Fl in polymer/ssDNA-Fl complex (Table 1). There is a competition between FRET and quenching (via eT) and eT can function as a major process at closer distance. Electron transfer is essentially a contact process with exponential distance (between donor and acceptor) dependence [6].

We measured the PL quantum yield (Φ) of Fl (in ssDNA-Fl) before and after electrostatic complexation with two polymers by switching to a more concentrated solution ([ssDNA-Fl] $= 10^{-6}$ M and [**P1i** or **P2i**] $= \sim 10^{-5}$ M). We observed the FRET spectra identical to those in Figure 2 at this concentration. The fluorescein PL Φ in ssDNA-Fl in the presence and absence of **P1i** or **P2i** was measured by exciting Fl directly. The Φ for ssDNA-Fl in water (at pH = 8) was determined to be 0.8. After polymer complexation ([**P1i** or **P2i**] $= 1.4 \times 10^{-5}$ M and [ssDNA-Fl] $= 10^{-6}$ M), the Φ values were measured to be 0.27 for **P2i**/ssDNA-Fl and ~ 0.01 for **P1i**/ssDNA-Fl. It suggests there is a quenching process by eT after electrostatic complexation for both **P1i**/ssDNA-Fl and **P2i**/ssDNA-Fl. After complexation with CCPs, almost complete PL quenching was observed for **P1i**/ssDNA-Fl and a relatively moderate quenching was measured for **P2i**/ssDNA-Fl. Most importantly the quenching is reduced by introducing the molecular bumper in **P2i**/ssDNA-Fl. Both **P1i** and **P2i** have same HOMO-LUMO electronic structures and a similar thermodynamic driving force for either FRET or eT. It appears that eT operates to a larger extent with **P1i**, proposing that the presence of the "molecular bumper" in **P2i** increases Fl emission by increasing the donor-acceptor distance, which decreases more acutely PCT quenching, relative to FRET.

It should be noted that the quantum yield decrease of the D/A complex due to eT from neighboring donors to the excited acceptor is a key factor to determine the efficiency in this detection scheme. Imagine that FRET efficiency is 100% but the acceptor after electrostatic complexation is not emissive. It does not function as a fluorescence DNA sensor. Molecular design strategies are necessary to prevent quenching of the acceptor emission in the complex as well as the efforts to improve the FRET efficiency. The intermolecular distance modulation using a molecular bumper is one example to show how to control it in the molecular scale for the optimized FRET DNA detection.

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