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Surfactant Effect on Energy Transfer between Cationic Conjugated Polymer and Dye-Labeled Oligonucleotide

*Kan-Yi Pu, Ruoyu Zhan, Bin Liu**

Summary: We report a convenient and effective method to enhance the signal output of dye-labeled oligonucleotide sensitized by cationic conjugated polymers (CCP). Sodium dodecyl sulphate (SDS) is utilized to regulate the interaction between CCP and dye-labeled single-stranded DNA in order to reduce the dye self-quenching within the CCP/DNA complexes. Improvement of CCP-sensitized dye emissison in the presence of SDS relative to that in the absence of SDS is observed, which reveals the importance of reducing CCP charge density in improving the energy transfer from CCP to dye-labeled probes.

Keywords: biosensor; conjugated polymer; energy transfer; surfactant

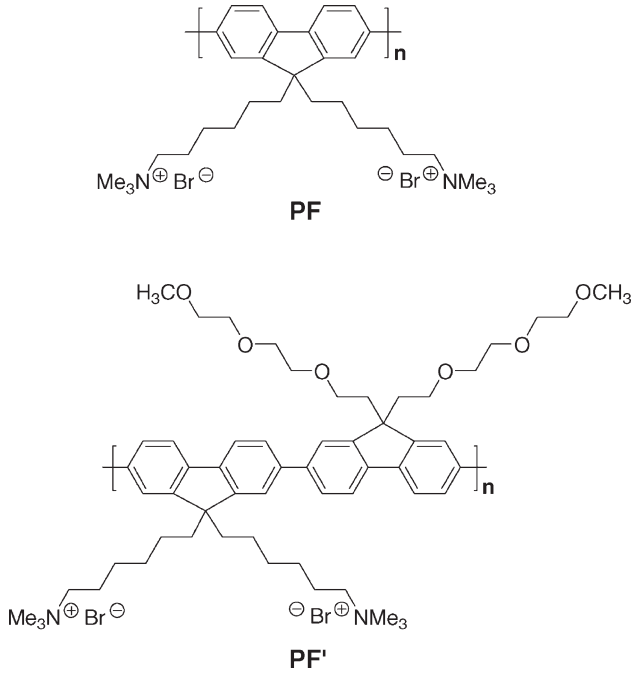
Introduction

Reliable methods for the detection of nucleic acids are under intensive investigations due to their vital applications in clinical diagnosis, environmental monitoring, forensic analysis and antiterrorism.[1] Cationic conjugated polymers (CCPs) have been utilized for DNA detction, which takes advantage of light-harvesting properties of CCPs to amplify the signal output of chromophore-labeled DNA or peptide nucleic acid (PNA).[2] The signal amplification upon excitation of CCPs, relative to that upon direct excitation of the chromophore, stems from efficient exciton migration from the electron-delocalized backbone of CCP (donor) to the chromophore (acceptor) through fluorescence resonance energy transfer (FRET).[3]

To improve the sensitivity of CCP-based DNA assays, a variety of CCPs have been developed to match the optimum conditions for FRET process.[2,4] Recently, we designed and synthesized two CCPs with different side chains,[5] where the charge density of **PF′** is half that of **PF** as shown in Scheme 1. The energy transfer experiments indicate that **PF′** is a better signal ampilifier than **PF**. The underlying mechanism is that **PF′** with the decreased charge density has weakened electrostatic attractions and loosened DNA compaction within the CCP/DNA complexes. This results in decreased local concentration of the chromophore within the complexes, ultimatly leading to suppressed chromophore self-quenching. This study highlights that a reduction in charge density of CCP gives rise to improved CCP-sensitized chromophore emission.

In this report, we show that anionic surfactant can be used to neutralize the positive charges of **PF** and in turn to improve the polymer-sensitized signal output of chromophore-labeled DNA. Sodium dodecyl sulphate (SDS) was chosen as the representative surfactant, while Texas Red labeled single-stranded DNA (ssDNA-TR) was used as the probe for this study. All experiments were conducted in water to exclude the effect of buffer ions on surfactant micelle structures as well as their interactions with **PF** and DNA.[6]

Department of Chemical and Biomolecular Engineering, 4 Engineering Drive 4, National University of Singapore, Singapore 117576, Singapore
E-mail: cheliub@nus.edu.sg



Scheme 1.
Chemical structures of **PF** and **PF′**.

Experimental Part

Methods

UV-vis spectra were recorded on a Shimadzu UV-1700 spectrometer. Fluorescence measurements were carried out on a Perkin Elmer LS-55 equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using 90 degree angle detection for solution samples. The UV and PL experiments were carried out at $25 \pm 1\,^{\circ}C$. MilliQ water (18.2 MΩ) was used for all the experiments.

Materials

HPLC-purified DNA oligonucleotide (5′-TR-ATC TTG ACT ATG TGG GTG CT-3′) was purchased from Research Biolabs, Singapore. **PF** was synthesized according to our previous report.[5]

Results and Discussion

Figure 1 shows the normalized PL spectrum of **PF** which has an emission maximum at 426 nm. The normalized absoprtion and PL spectra of TR in water is also shown in Figure 1. TR has absorption and emission maxima at 595 and 615 nm, respectively. The emission tail of **PF** extending to 575 nm overlaps with the TR absorption, ensuring the feasibility of energy transfer from **PF** to TR.

Energy transfer experiments from **PF** to ssDNA-TR were conducted in water at

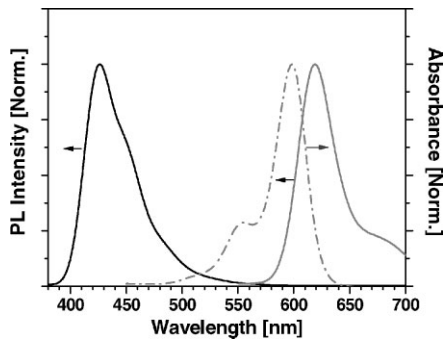


Figure 1.
Normalized PL spectra (solid line) of **PF** (black) and TR (gray) and the absorption spectrum of TR (gray dashed line) in water.

[ssDNA-TR] = 20 nM in the absence and presence of SDS at [SDS] = 9 μM. The excitation wavelength was fixed at 390 nm where TR has almost no absorption. Hence, the resulted TR emission is mainly ascribed to FRET from **PF**. The used SDS concentration was optimized for good FRET from **PF** to ssDNA-TR. In these experiments, **PF** was gradually added into the ssDNA-TR solution until the TR emission was saturated upon excitation of **PF**. The concentration of **PF** is calculated based on the repeat unit (RU). Figure 2 summarizes the **PF**-sensitized TR emission intensity as a function of [RU] in the presence and absence of SDS. For both curves, the TR intensity gradual increases with increasing [RU], which is followed by saturation at [RU] = 400 and 700 nM for that in the absence and presence of SDS, respectively. At each [RU], the TR intensity in the presence of SDS is higher than that in the absence of SDS.

Figure 3 shows the **PF**-sensitized TR PL spectra in water at [ssDNA] = 20 nM in the presence of SDS at [RU] = 700 nM and in the absence of SDS at [RU] = 400 nM upon excitation at 390 nm. The **PF′**-sensitized TR PL spectrum in water at [ssDNA-TR] = 20 nM and [RU] = 700 nM upon excitation at 390 nm is also given in Figure 3 for comparison. It can be found that the **PF**-sensitized TR emission intensity in the presence of SDS is ~2-fold of that in the absence of SDS, and is slightly larger than the **PF′**-sensitized TR emission intensity. These data indicate that introduction of SDS into the FRET system can enhance the polymer-sensitized chromophore emission, analogous to the observed effect of reduced charge density of **PF′** *vs* **PF** on energy transfer.

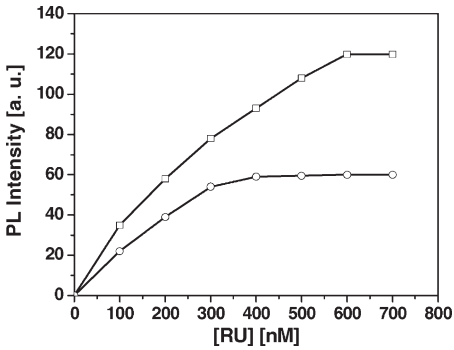


Figure 2.
PF-sensitized TR emission intensity as a function of [RU] in the absence (circles) and presence of SDS (squares) at [SDS] = 9 μM.

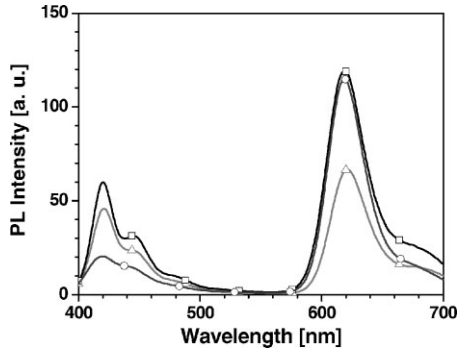


Figure 3.
PL spectra of **PF**/ssDNA-TR in the absence of SDS (triangles) at [RU] = 400 nM and in the presence of SDS at [SDS] = 9 μM (squares) and [RU] = 700 nM in water. The PL spectrum of **PF′**/ssDNA-TR at [ssDNA-TR] = 20 nM and [RU] = 700 nM (circles) in water is also shown for comparison. [ssDNA-TR] = 20 nM, excitation at 390 nm.

To understand the origin of SDS-enhanced **PF**-sensitized TR emission, the intrinsic PL spectra of TR upon addition of **PF** into ssDNA-TR solution at [ssDNA-TR] = 20 nM in the presence and absence of SDS at [SDS] = 9 μM were monitored upon excitation at 595 nm. The obtained PL spectra are analyzed using the Stern-Volmer method. The Stern-Volmer equation can be applied to quantify a quenching process:[7]

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

Where, I_0 and I are defined as the emission intensity in the absence and presence of the quencher, respectively; [Q] is the concentration of the quencher. K_{SV} is the Stern-Volmer constant, which provides a quantitative measure of quenching efficiency. Figure 4 shows the Stern-Volmer plots of ssDNA-TR quenched by **PF** in the linear range, in the presence of 9 μM SDS and in the absence of SDS. The *K*sv values

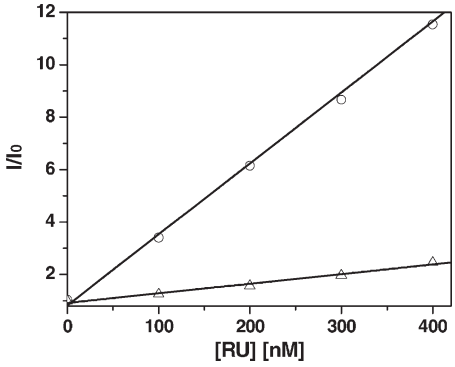


Figure 4.
The Stern-Volmer plots of ssDNA-TR quenched by **PF** in the presence and absence of SDS at [SDS] = 9 μM.

are calculated to be 3.7×10^6 and 2.7×10^7 M^{-1} in the presence and absence of SDS, respectively. The decreased quenching constant in the presence of SDS indicates that TR fluorescence quenching by **PF** is reduced by SDS.

The decreased TR quenching in the presence of SDS should stem from the SDS-reduced charge density of **PF** through the charge neutralization process, which subsequently leads to weakened DNA compaction within the **PF**/ssDNA-TR complexes. As such, introduction of SDS into the **PF**/ssDNA-TR FRET system improves the polymer-sensitized dye emission, which is consistent with our previous finding that a reduction in the charge density of CCP is beneficial to the polymer-sensitized dye emission.[5]

Conclusions

We have demonstrated that introduction of SDS into the **PF**/ssDNA-TR system can effectively reduce TR quenching upon **PF**/ssDNA-TR complexation, ultimately leading to enhanced **PF**-sensitized TR emission. The surfactant effect on FRET is comparable to the synthetic methodology wherein the charge density of CCP is decreased by copolymerization. This study highlights that optimization of the interaction between water-soluble conjugated polymers and dye-labeled probes plays an important role in improving the signal amplification of CCP-based biosensors.

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