Langmuir
Letter
pubs.acs.org/Langmuir

Intercalation-FRET Biosensor with a Helical Conjugated Polyelectrolyte

Eunkyung Ji, Danlu Wu, and Kirk S. Schanze*

Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200

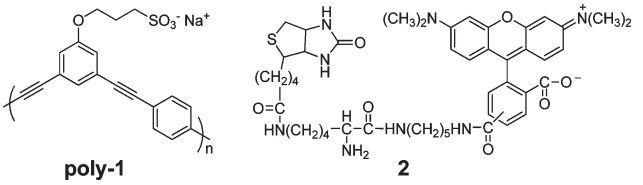
Received July 30, 2010

A biotin-tetramethylrhodamine (biotin-TMR) quencher-ligand interacts with a (phenylene-ethynylene) based helical conjugated polyelectrolyte (**poly-1**) via intercalation of the TMR unit into the helix. The interaction is signaled by efficient fluorescence resonance energy transfer (FRET) from the polymer to the TMR chromophore. Avidin addition to the **poly-1**/biotin-TMR intercalation complex does not interrupt FRET, instead resulting in the formation of avidin−biotin "cross-links". Mixing of biotin-TMR with avidin prior to addition of the polymer efficiently disrupts the FRET signal, giving rise to a sensor with a detection limit of 100 pM for avidin. Study of the FRET response as a function of biotin-TMR and avidin concentration affords insight into the interaction of the protein with the **poly-1**/biotin-TMR intercalation complex.

Conjugated polyelectrolytes (CPEs) have been extensively studied as fluorescent sensors due to their ability to undergo efficient "amplified quenching" by oppositely charged quencher ions.[1−6] Since the first report of an avidin sensor based on reversible fluorescence quenching of an anionic poly(phenylene vinylene) (PPV) CPE by a biotin-viologen "quencher-ligand",[1] several groups have reported analogous CPE fluorescent sensors for the biotin−avidin interaction.[7−9] A recent study challenged the early work on the PPV/biotin-viologen sensor by demonstrating that the anionic CPE is subject to nonspecific interactions with avidin and other hydrophobic proteins.[10]

Previously, we reported the ability of a meta-linked, anionic poly(phenylene ethynylene) (**poly-1**, Chart 1) to adopt a helical conformation and serve as a host for species that can intercalate between π-stacked phenylene units in the helical assembly.[11,12] Several cationic chromophores that are known to intercalate into ds-DNA were shown to exhibit identical spectroscopic effects (e.g., absorption hypochromism and luminescence enhancement) upon binding to **poly-1**. For example, intercalation of $Ru(bpy)_2(dppz)^{2+}$ into **poly-1** leads to strong quenching of the polymer's fluorescence and an 80-fold enhancement of the luminescence from the Ru complex metal-to-ligand charge transfer state. The results are consistent with intercalation of the dppz ligand into the π-stacked phenylene units in helical **poly-1**.[13]

Chart 1



In the present Letter, we report a novel fluorescence resonance energy transfer (FRET) sensor based on **poly-1**/intercalation coupled with the biotin−avidin interaction. In particular, the tetramethylrhodamine (TMR) chromophore in quencher-ligand **2** intercalates into **poly-1**, quenching the polymer's fluorescence efficiently and giving rise to FRET enhanced TMR fluorescence at longer wavelength. However, when **2** is premixed with avidin, the interaction of **2** with **poly-1** is interrupted, and polymer quenching and FRET is strongly suppressed. This system has a detection limit for avidin of 100 pM, and the sensor operates in aqueous buffer solution. Importantly, the behavior of this sensor as a function of avidin/**2** ratio gives insight into the interaction of **2** with **poly-1**. The novelty of this work lies in the unique nature of the intercalation binding of **2** with **poly-1**, and how it influences the sensor response to avidin. Unlike other CPE based avidin sensors, the **poly-1/2** sensor retains its sensitivity in solutions with moderate ionic strength because the quencher ligand/polymer interaction involves hydrophobic and molecular recognition, in addition to electrostatic binding.

In aqueous phosphate buffer, **poly-1** absorbs in the near-UV (320 nm) and it exhibits a broad, structureless fluorescence band with $\lambda_{max} \sim 450$ nm. As reported previously, in water, **poly-1** is folded in a helical conformation, and the broad "excimer-like" emission arises due to $\pi-\pi$ interactions between phenylene-ethynylene units that are in close proximity in the helix. The TMR chromophore in quencher-ligand **2** absorbs at $\lambda_{max} \sim 553$ nm and is weakly fluorescent in water. There is good overlap between the **poly-1** fluorescence and **2** absorption, giving rise to the probability for efficient **poly-1** → **2** FRET.[14,15]

Figure 1 illustrates the changes in fluorescence that occur concomitant with addition of **2** to an aqueous buffer solution of **poly-1**. The fluorescence of **poly-1** at 450 nm is quenched

*To whom correspondence should be addressed. E-mail: kschanze@chem.ufl.edu.

(1) Chen, L. H.; McBranch, D. W.; Wang, H. L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287–12292.
(2) Wang, D. L.; Wang, J.; Moses, D.; Bazan, G. C.; Heeger, A. J. *Langmuir* **2001**, *17*, 1262–1266.
(3) Pinto, M. R.; Schanze, K. S. *Synthesis* **2002**, 1293–1309.
(4) Jiang, H.; Zhao, X. Y.; Schanze, K. S. *Langmuir* **2006**, *22*, 5541–5543.
(5) Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339–1386.
(6) Jiang, H.; Taranekar, P.; Reynolds, J. R.; Schanze, K. S. *Angew. Chem., Int. Ed.* **2009**, *48*, 4300–4316.
(7) O'Connell, M. J.; Chan, C. K.; Li, W. G.; Hicks, R. K.; Doorn, S. K.; Wang, H. L. *Polymer* **2007**, *48*, 7582–7589.
(8) Pinto, M. R.; Tan, C.; Ramey, M. B.; Reynolds, J. R.; Bergstedt, T. S.; Whitten, D. G.; Schanze, K. S. *Res. Chem. Intermed.* **2007**, *33*, 79–90.
(9) Song, X. D.; Wang, H. L.; Shi, J.; Park, J. W.; Swanson, B. I. *Chem. Mater.* **2002**, *14*, 2342–2347.
(10) Dwight, S. J.; Gaylord, B. S.; Hong, J. W.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 16850–16859.
(11) Tan, C. Y.; Pinto, M. R.; Kose, M. E.; Ghiviriga, I.; Schanze, K. S. *Adv. Mater.* **2004**, *16*, 1208–1212.
(12) Zhao, X. Y.; Schanze, K. S. *Langmuir* **2006**, *22*, 4856–4862.
(13) Friedman, A. E.; Chambron, J. C.; Sauvage, J. P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960–4962.
(14) Forster, T. *Ann. Phys.* **1948**, *2*, 55–75.
(15) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer Verlag: 2006.

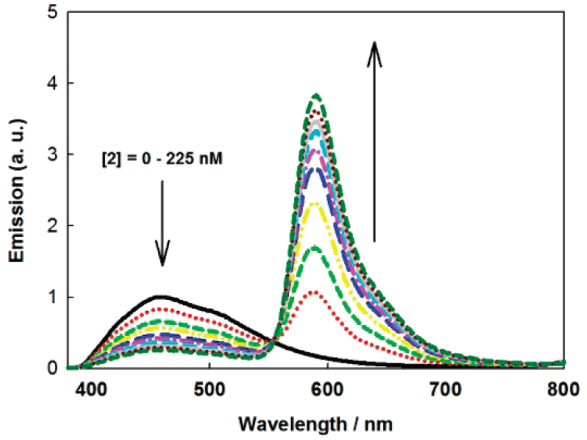


Figure 1. Normalized fluorescence spectra (λ_{ex} = 320 nm) of **poly-1** (c = 15 μM) upon addition of **2** (c = 0−225 nM) in aqueous phosphate buffer (1 mM, pH 7.4). Arrows show direction of change of the bands with increasing [**2**].

(with K_{SV} = 1.1 × 10^7 M^{-1}), and strong fluorescence appears at λ_{max} ~ 590 nm from **2**. Importantly, under the same excitation conditions, the fluorescence from **2** alone is negligible (Supporting Information Figure S-1), which indicates that the emission seen from the **poly-1**/**2** complex is due to FRET. The excitation spectrum of the **poly-1**/**2** complex demonstrates that efficient energy transfer occurs from **poly-1** to **2** (Supporting Information Figure S-2), confirming the FRET hypothesis. Fluorescence excitation anisotropy confirms that **2** is in a confined environment in which rotational motion is restricted in the **poly-1**/**2** complex, and that the absorption and emission dipoles of the polymer and dye are not parallel.[15−17]

Analogous fluorescence quenching studies were carried out with **2** and $PPESO_3^-$. This polymer has a similar repeat unit structure as **poly-1**, but it is linear due to all para- linkages in the phenylene-ethynylene backbone.[18] In the $PPESO_3^-$/**2** system, quenching is less efficient (K_{SV} = 6.3 × 10^6 M^{-1}), and sensitized emission from the TMR chromophore is lacking, indicating that FRET does not occur. This comparative study underscores the unique nature of the interaction between helical **poly-1** and **2**, supporting the hypothesis that the interaction is dominated by intercalation of the TMR chromophore into the **poly-1** helix.

A key component of this study is to examine the effect of the biotin−avidin interaction[19] on FRET between **poly-1** and **2**. Initially, we expected that addition of avidin to the preformed **poly-1**/**2** complex would elicit a strong sensory response, as the avidin was expected to interrupt FRET, by binding to the biotin unit and thus disrupting the intercalation of **2**. Surprisingly, addition of avidin to the preformed intercalation complex between **poly-1** and **2** gives rise to little response. The fluorescence of **2** is slightly quenched by avidin; however, recovery of **poly-1** fluorescence does not occur. This result suggests that avidin is unable to disrupt the intercalation of **2** into **poly-1**, but rather the protein leads to the formation of a biotin−avidin complex "cross-link" with the **poly-1**/**2** complex.[10,20] FRET is not disrupted in this complex because the rhodamine chromophore

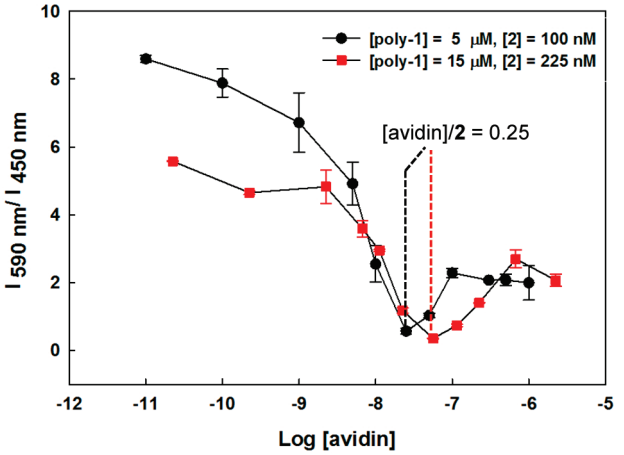
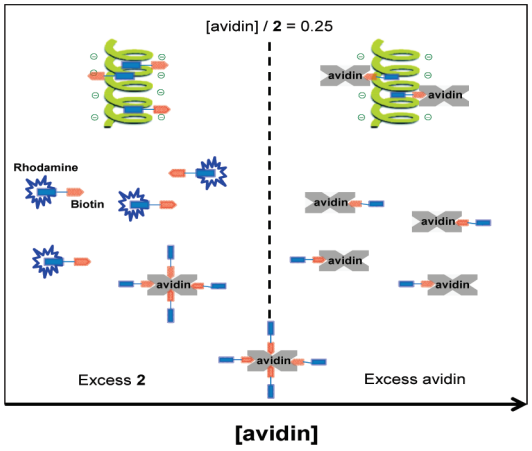


Figure 2. Ratio of intensities at 590 and 450 nm after addition of premixed **2**−avidin complex at various avidin concentrations in phosphate buffer (1 mM, pH 7.4), λ_{ex} = 320 nm. For [**2**] = 100 nM, the analytical detection limit of avidin is 100 pM.

Scheme 1. Binding of Preformed 2−Avidin Complex to Poly-1 as a Function of Added Avidin Concentration



remains intercalated.[21] The formation of cross-links induced by avidin addition is evidenced by studies using fluorescence correlation spectroscopy (FCS).[22] These experiments reveal that addition of avidin to the **poly-1/2** complex leads to formation of fluorescent aggregates with a hydrodynamic radius that is more than 20-fold larger than that of either the **poly-1/2** or **2**-avidin complexes.

By contrast, a strong sensor response is observed when avidin and **2** are mixed prior to addition of **poly-1**. Figure 2 shows the sensor response as a function of avidin concentration, where FRET efficiency is indicated by the ratio of the fluorescence intensity at 590 and 450 nm (I_{590}/I_{450}). Experiments performed for two different concentrations of **2** show that FRET decreases with increasing avidin concentration, reaching a minimum at [avidin]/[**2**] = 0.25 and then increasing again as the concentration of avidin increases further. Note that the minimum FRET occurs at [avidin]/[**2**] = 0.25 for both concentrations of **2** examined, indicating that the 1:4 avidin−**2** complex is least able to interact with and undergo FRET from **poly-1**. Weak interaction of the 1:4 avidin−**2** complex with **poly-1** is likely due to steric constraints which prevent intercalation of the avidin bound **2**.

Scheme 1 suggests the possible reason for the complex dependence of FRET on the [avidin]/[**2**] ratio. First, for [avidin]/**2** < 0.25, all

(16) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446–5451.
(17) Fluorescence anisotropy values (r) for excitation at wavelengths corresponding to absorption of the TMR chromophore and the polymer are r = 0.15 and −0.05, respectively.
(18) Tan, C. Y.; Pinto, M. R.; Schanze, K. S. *Chem. Commun.* **2002**, 446–447.
(19) Livnah, O.; Bayer, E. A.; Wilchek, M.; Sussman, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5076–5080.
(20) Slim, M.; Durisic, N.; Grutter, P.; Sleiman, H. F. *Chembiochem* **2007**, *8*, 804–812.
(21) The weak quenching that is observed arises due to quenching by avidin (ref 7). It was demonstrated in a separate experiment where adding avidin leads to the fluorescence quenching of **2** in the absence of **poly-1**.
(22) Wu, D.; Chen, Y.; Tan, W.; Schanze, K. S. Unpublished results.

avidin is in the 1:4 complex and excess **2** is able to bind to **poly-1**, giving rise to FRET. An increase in avidin concentration decreases the available **2**, thus giving rise to the observed decrease in FRET. However, when [avidin]/**2** > 0.25, all of the **2** is bound to avidin, but because the protein is in excess, an increasing number of avidin−**2** complexes have less than four **2** bound per protein. Apparently, these lower occupancy avidin−**2** complexes are able to more effectively interact with **poly-1**, perhaps allowing intercalation of the TMR chromophore (Scheme 1), thus giving rise to the increased FRET signal.

In summary, we describe the application of a fluorescent, helical CPE as the "transducer" for an avidin−biotin fluorescent sensor. The sensor response is based on FRET between an intercalated dye and the helical CPE, and interruption of this interaction by the avidin−biotin interaction. The sensor response is high in buffer solution, because the quencher-ligand/CPE binding relies on molecular recognition/hydrophobic interactions, as opposed to solely electrostatic interactions which are common for other CPE based sensor systems.

Acknowledgment. We acknowledge the National Science Foundation for support of this work (Grant No. CHE-0515066).

Supporting Information Available: Experimental details, fluorescence spectra of **2** in the absence and presence of **poly-1**, excitation spectrum of **poly-1**/**2** complex along with absorption spectra of **poly-1** and **2**, and fluorescence spectra for $PPESO_3^-$/**2** quenching study system. This material is available free of charge via the Internet at http://pubs.acs.org.