Forced Agglutination as a Tool to Improve the Sensory Response of a Carboxylated Poly(*p*-phenyleneethynylene)

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ABSTRACT: A water-soluble biotinylated poly(*p*-phenyleneethynylene) (PPE, **3**) was synthesized by postfunctionalization of an amine−PPE precursor **2a** and investigated for sensory responses in aqueous buffer at physiological pH. Polymer **3** shows similar sensitivity ($K_{SV} = 1.1 \times 10^4$ M^{-1}) to Hg^{2+} ions when compared to PPE **1** ($K_{SV} = 1.3 \times 10^4$ M^{-1}); **3** was exposed to streptavidin-coated microspheres (SCM) to form a self-assembled complex, which exhibits an enhanced sensitivity toward Hg^{2+} ions. If **3** was exposed to either avidin, a streptavidin−rhodamine conjugate, or a streptavidin−Texas Red conjugate, fluorescence of **3** was quenched with K_{SV} values of 33×10^6, 28×10^6, and 9×10^6 M^{-1}, respectively. When lightly cross-linked polymer arrays **3**−avidin were exposed to Hg^{2+} ions, they were more sensitive than **3** alone. The complex was quenched by Hg^{2+} ions with a K_{SV} of 1.1×10^5 M^{-1}. The negatively charged PPE **1** was incubated with the positively charged avidin to form an electrostatic **1**−avidin complex. The **3**−avidin complex was about 10 times as sensitive to Hg^{2+} ions as **1** and 25 times as sensitive to Hg^{2+} as **1**−avidin.

Introduction

Grafting of suitable recognition units to the side chains of conjugated polymers (CP) provides bio- and chemosensory materials. CPs exhibit molecular wire behavior, multivalent display of recognition units, and superquenching effects all intertwined with spectroscopic properties that stem from conformational changes, excimer/exciplex formation, and fluorescence resonance energy transfer.[1−6] Materials with exquisite sensitivity and selectivity for specific analytes can result. If CPs are appended with ionic or highly polar side groups, sensing of environmentally and biologically important species is possible in aqueous solution. Current applications involving water-soluble CPs are recent DNA−sensors, CP−protein complexes, and sugar-coated poly(*p*-phenyleneethynylene)s (PPE)s for the detection of *E. coli*.[7−11]

We have explored water-soluble PPE−protein complexes as agglutination assays for mercury ions, exploiting the sulfhydryl−protease papain as a cofactor, which increased the sensory response of PPE **1** by a factor of 20 toward Hg^{2+} ions.[12,13] In this contribution, we investigate the influence of preaggregation of a biotinylated PPE, **3**, with avidin or streptavidin upon its response to mercuric ions and paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride).[14] While numerous biotin-functionalized PPEs have been synthesized and examined in their binding ability to (strept)avidin,[10,14] this contribution goes a step further as it explores the sensory response of the biomolecular constructs obtained by mixing of a biotin−PPE with (strept)avidin; this concept should allow us to modulate and hone the properties of CPs toward desired ends. We were inspired by Schanze's work, demonstrating that aggregation of a *meta*-PPE positively influences its sensitivity toward quenching agents, suggesting that aggregated CPs act as coupled electronic entities.[15] We set out to emulate the aggregation by exposing a biotinylated PPE, **3**, toward avidin and exploit the properties of the force-aggregated PPE for sensory applications.

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Results and Discussion

Synthesis of the biotin-functionalized PPE **3** was performed by 1-ethyl-3-(3′-(dimethylamino)propyl)carbodiimide (EDC)[16] mediated postfunctionalization (Scheme 1) of **2a** (SI) with D-biotin in anhydrous DMF. The water-soluble, biotin-functionalized PPE **3** was obtained after hydrolysis of the ester groups by sodium hydroxide in methanol. The structure of **3** was secured by ^{1}H NMR spectroscopy, where the biotin units display their specific signals at $\delta = 2.85-1.91$ ppm in addition to the expected bands for the PPE backbone.

The Stern−Volmer equation is useful when measuring the quenching of a conjugated polymer by a quencher Q. The Stern−Volmer constant K_{SV} is defined by[17,18]

$$F_0/F = K_{SV}[Q] + 1$$

In this equation, F_0 is the fluorescence intensity without added quencher Q, and F is the fluorescence intensity in the presence of Q at a given concentration [Q]. A higher value of K_{SV}, the slope, indicates a greater sensitivity of the system toward Q. Stern−Volmer quenching is static if Q forms a ground-state complex with the fluorophore; K_{SV} represents the binding constant between the quencher and the fluorophore. Alternatively, in dynamic or collisional quenching, Q deactivates the excited state of the fluorophore. Dynamic quenching is unlikely in PPEs, where fluorescence lifetimes of the fluorophore under consideration are short (300−400 ps). Quenching processes for PPEs are generally accepted to be static in nature. The Stern−Volmer constant, K_{SV}, is formally independent upon the concentration of the fluorophore; experimentally, however, it is found that K_{SV} can decrease with increasing concentration of the CP under investigation.[19]

Table 1 shows the results of the quenching experiments performed with **1**−**3**. The first five entries show controls that were performed with **1**, which does not promote any specific interaction between polymer and avidin or strepavidin. Yet, nonspecific interactions between avidin and **1** are strong, similar to the cases of protein−CP interactions that have been reported

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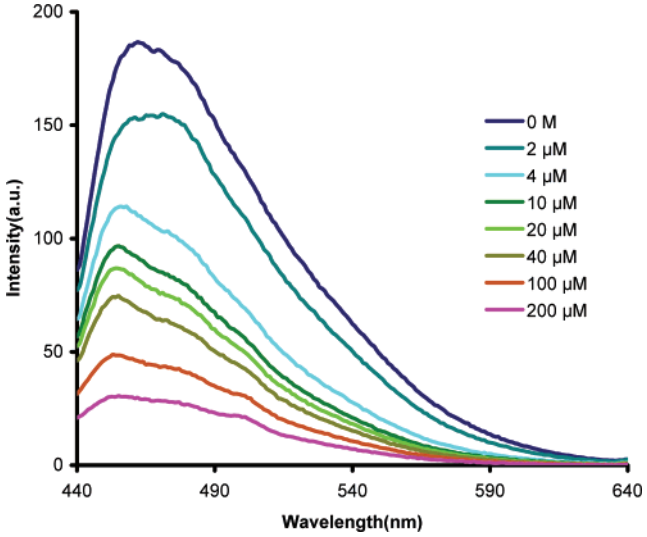


Figure 1. Emission spectra of solutions of lightly cross-linked polymer arrays from **3** (1 mg L^{-1}) and avidin (60 nM) by addition of increasing concentrations of mercury ions.

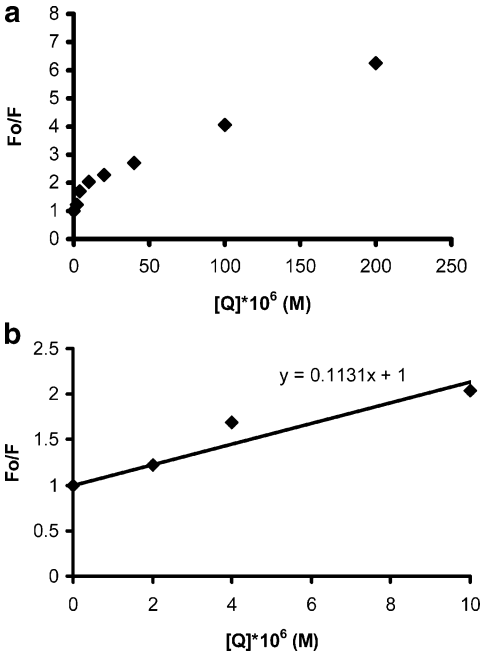


Figure 2. (a) F_0/F plots for **3**−avidin "armed" polymer with Hg^{2+} ions, and (b) the apparent Stern−Volmer constant (K_{SV}) for Hg^{2+} ions based on initial linear parts is 1.1×10^5 M^{-1}.

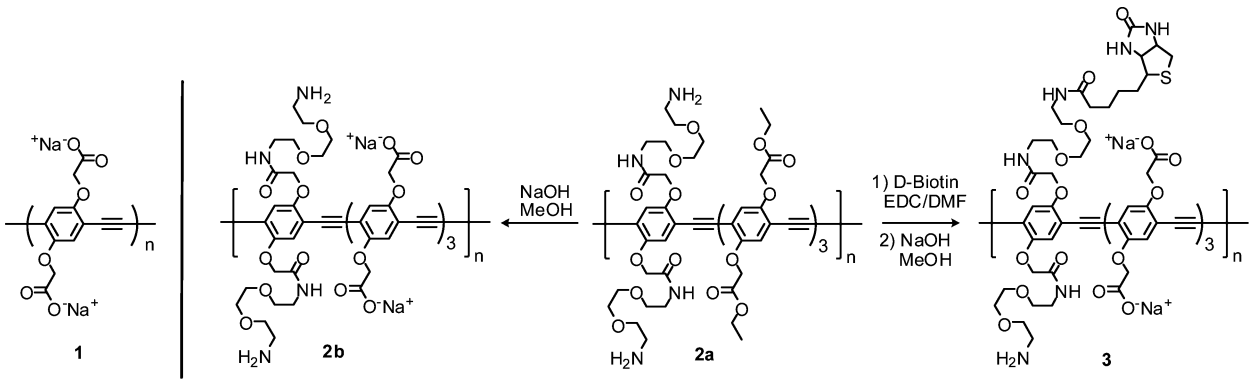
recently.[13] The specific interactions between **3** and avidin, streptavidin, or dye-labled streptavidin give K_{SV} values that range from 0.9 to 3.3×10^7 M^{-1}. While these K_{SV} values are quite high, they are only by a factor of 10 higher than those that were found for the nonspecific interaction of **1** with avidin. They do not reflect the very high ($K_{assoc} \sim 10^{15}$) binding constant of (strept)avidin to biotin. The reason for the apparently diminished binding might be the insufficient lengths of the connecting tether that chains the biotin to the backbone. Control experiments in which **3** or its avidin/streptavidin complex were exposed toward paraquat only showed marginal K_{SV} values due to the high ionic strength (see Supporting Information) at which the experiments were performed, using 0.1 M buffer solutions.

The measured K_{SV} values, which indicate the complex formation between the polymers **1**−**3** and mercury, are between $K_{SV} = (0.8-1.3) \times 10^4$ M^{-1}. The three polymers behave quite similarly, which is somewhat unexpected but shows that the amino-substituted oligoethylene glycol groups and the carboxylate units must have similar innate binding affinities toward the mercuric ion.

The self-assembled complexes between either **1** and avidin or **3** and avidin or streptavidin are compared in their sensitivity toward mercury ions. The self-assembled complex of **1** and avidin binds *less* to mercuric ions ($K_{SV} = 4 \times 10^3$) than **1** by itself, while in the case of the biotinylated PPE **3**, preagglutination with either streptavidin or avidin leads to a significant increase in the binding of the complex to mercuric ions. The highest K_{SV} of 1.1×10^5 M^{-1} resulted when the complex of **3**−avidin was exposed to mercuric ions (Table 1, entry 15), representing an almost 10-fold increase of K_{SV} compared to the binding of mercuric ions to **3**.

We find that PPEs **1** and **3** are moderately quenched by mercuric ions ($K_{SV} \approx 10^4$ M^{-1}) in buffered aqueous solution. Methyl viologen quenches the fluorescence of **3** only weakly with a K_{SV} of 1.7×10^3 M^{-1}. This relatively low K_{SV} is due to the highly ionic environment in which the quenching experiments are performed. Upon addition of avidin, streptavidin, or streptavidin-coated microspheres, the quenching of **3** by methyl viologen is not more efficient, suggesting that the proteins, while agglutinating **3**, may as well screen the polymer chains further from paraquat. In the case of mercuric ions, the situation is different, and here a 10-fold increase in sensitivity is observed when we compare the quenching of **3** to that of the **3**−avidin complex. Interestingly, self-assembled electrostatic complexes formed from **1** and avidin show a *decreased* binding of mercury when compared to the binding of mercuric ions to **1** alone. We assume that the negative charges of **1** are partly neutralized by the presence of avidin and do not allow for mercuric ions to bind tightly. In the case of the **3**−avidin complex, the significant

Scheme 1. Synthesis of PPE 2b and Biotin-Functionalized PPE 3 by Postfunctionalization of 2a; Structure of Carboxylate PPE 1 is Shown



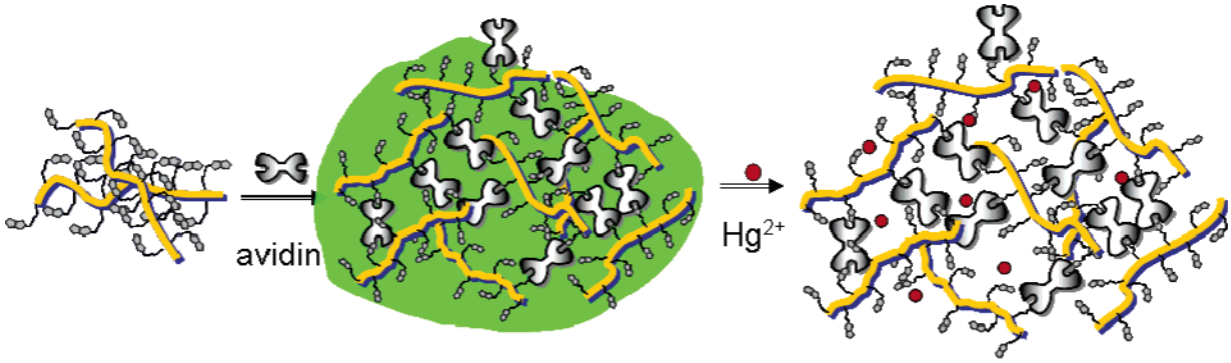


Figure 3. Proposed mechanism of the quenching effects shown by the **3**−avidin or streptavidin agglutinates upon addition of mercuric ions.

Table 1. Measured Stern−Volmer Constants K_{SV} (M^{-1}) for the Quenching of PPEs (1−3)

entry	substrate	cofactor	quencher	K_{SV}	comment
1	**1**		mercuric ion	1.1×10^4	
2	**1**		avidin	2.3×10^6	slope obtained from very low [Q]
3	**1**		SA−Rhodamine[a]	no quenching	
4	**1**		SA−Texas Red[a]	no quenching	
5	**1**	avidin	mercuric ion	4×10^3	
6	**2b**		mercuric ion	8×10^3	
7	**3**		SA-coated microspheres	qualitiative quenching	
8	**3**		avidin	3.3×10^7	slope obtained from very low [Q]
9	**3**		SA−Texas Red[a]	9×10^6	
10	**3**		SA−Rhodamine[a]	2.8×10^7	
11	**3**		mercuric ion	1.3×10^4	
12	**3**		paraquat	1.7×10^3	low, due to high ionic strength
13	**3**	SA-coated microspheres[a]	mercuric ion	7.2×10^4	
14	**3**	SA[a]	mercuric ion	5.5×10^4	
15	**3**	avidin	mercuric ion	1.1×10^5	
16	**3**	avidin	paraquat	1.5×10^3	low, due to high ionic strength
17	**3**	SA[a]	paraquat	2.3×10^3	low, due to high ionic strength

[a] SA = streptavidin.

length of the linker, while allowing for an effective interaction of biotin with the avidin and therefore cross-linking, will keep the polymer chains sufficiently far away from the protein and allow interaction of the carboxylate anions with the mercuric ions.

What is the proposed mechanism for the enhanced quenching of the **3**−avidin complex when compared to **3**? In the first step, a complex between the biotinylated PPE and avidin (**3**−avidin) is formed (Figure 3); the fluorescence of this complex decreases. Even very high concentrations of avidin, however, were never able to diminish the fluorescence of **3** (or its complex) to less than 40% of the starting value. The **3**−avidin complex must enforce a significant degree of interaction between the polymer chains short of an excimer or exciplex. The addition of mercuric ions leads to efficient quenching of the fluorescence of **3**−avidin. The concept of using preagglutination is of significance as it suggests that the very loose network of CP chains, formed by self-assembly, using the biotin−avidin interactions, is primed to be more sensitive to specific analytes than isolated polymer chains are. In the proposed three-dimensional multiplex, the excited-state energy transfer is more facile than in only one dimension (i.e., along one chain). In effect, a "3-d molecular wire" results, the fluorescence of which is efficiently quenched by the addition of mercuric ions.

There is precedence for this behavior. In Langmuir−Blodgett films of PPEs, where enhanced energy transport was observed in two dimensions.[22] In our case, the loose 3-d network is formed in solution and leads to the desired, enhanced sensory response. While the amplification factor is substantial, manipulation of the side chains to increase the avidin−biotin binding with the use of longer polymers (higher degree of polymerization) and linkers of increased length should lead to further significant gains in sensitivity.

Conclusions

We have synthesized the water-soluble biotinylated PPE **3** and examined its metal responsive behavior. Upon addition of avidin, **3** forms a complex, which displays increased sensitivity toward Hg^{2+} ions when compared to **3** alone. We have effectively "armed " **3** by avidin to obtain a substantial gain in sensitivity when monitoring for the presence of mercuric ions. While the arming of the biotinylated polymer by (strept)avidin is nonspecific with respect to mercuric ions, it increases the *sensitivity* of **3** toward Hg^{2+}, a conceptually important process. On the other hand, when the positively charged avidin forms an electrostatic complex with **1**, it *prevents* the quenching of the fluorescence of **1** by Hg^{2+} ions, probably due to the blockage of the carboxylate groups by the positively charged avidin.

The lightly cross-linked **3**−avidin complex is proof that CPs display a significantly increased sensitivity toward mercuric ions if self-assembly processes are used to form large soluble arrays, here promoted by biotin−(strept)avidin interactions. The result is an ensemble of electronically coupled polymer chains. We will further study such constructs to increase the sensitivity of

sugar-substituted and other PPEs, useful for the detection of lectin-based toxins and bacteria such as *E. coli*.

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Supporting Information Available: Synthetic details including NMR and UV−vis characterization of monomers **2**−**4** and details for the sensing experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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