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Modulating the Sensory Response of a Conjugated Polymer by Proteins: An Agglutination Assay for Mercury Ions in Water

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Agglutination assays are tools of microbiology and bioanalytical chemistry that identify blood groups, antigen−antibody interactions, prion proteins, and bacteriae such as *S. aureus*.[1,2] Herein, we describe an agglutination assay for Hg^{2+} as proof of principle for protein-mediated fluorescence modulation of a poly(*para*-phenyleneethynylene) (PPE) **1**. This concept should be broadly applicable and be restricted to neither PPEs nor the proteins we have investigated herein.

Functionalized PPEs are used to detect transition-metal ions, sugar binding proteins (lectins), bacteriae, etc.[3−6] Schanze et al. and our group have reported upon **1**;[7,8] its solutions are precipitated by the addition of Pb^{2+} [8] but not by Hg^{2+}, which leads to weak fluorescence quenching of **1** in PIPES (piperazine-1,4-bis(2-ethanesulfonic acid)) buffer at pH 7.2.

How would other metal ions react with **1**? A solution of **1** (5 μM/repeat unit) in phosphate buffer (0.1 M, pH 7.2)[8b] was exposed to solutions of 10 different metal ions (0.4 mM). The top panel of Figure 1 shows the results of this experiment when the vials are illuminated by a hand-held UV light (λ_{max} = 365 nm). At low concentrations of **1**, Pb^{2+} ions show slight quenching of the fluorescence of **1** but no precipitation; Zn^{2+}, Fe^{2+}, and Ni^{2+} also quench the fluorescence of **1**, whereas Ca^{2+} ions induce an aggregation induced red shift of the fluorescence of **1**.[9] The other investigated cations (Co^{2+}, Cu^{2+}, Mg^{2+}, Cd^{2+}) do not elicit a significant response from **1**. Mercury ions (0.4 mM) show the greatest quenching effect on the fluorescence of **1**. Could the addition of a cofactor increase the sensitivity of **1** toward Hg^{2+}?

We speculated that the positively charged papain, known to bind to Hg^{2+}, would form an electrostatic complex with **1** in solution. The fluorescence of **1**·papain might be sensitive toward Hg^{2+} ions because of the presence of the free sulfhydryl groups in the **1**·papain complex.[8c,10,11d] Proteins modulate the emissive properties and aggregation behavior of **1** and other conjugated polymers.[12] Although we expected papain to be an effective cofactor, we wanted other **1**·cofactor complexes (**1**·histone, **1**·bovine serum albumin (BSA), **1**·poly(dimethyldiallylammonium chloride) (Pdac, **2**)) as controls. Pdac **2** is a positively charged polyelectrolyte. Histone is a compact, positively charged protein used to wrap DNA, and BSA is a negatively charged serum protein used for the biological trafficking of hydrophobic species. We prepared a series of stock solutions where **1** (5 μM/repeat) was codissolved with Pdac (**2**) (25 μM/repeat), histone (5 μM), BSA (5 μM), and papain (5 μM), respectively.

The addition of **2** to **1** leads to a red shift and an increase of fluorescence of **1**; **2** wraps around **1**, which leads to planarization of the chains and at the same time suppresses excimer formation. The local increase in the refractive index in the complex **1**·**2** will give an additional red shift. The **1**·**2** complex is insensitive to the presence of most metal cations; only Fe^{3+} leads to slight quenching. Addition of BSA increases the fluorescence of **1**, whereas the fluorescence of **1** is somewhat quenched by histone and papain.[12a]

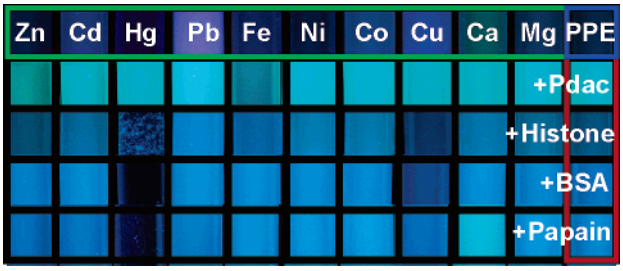

Figure 1. Addition of metal ions to PPE **1** in the absence and the presence of cofactors. Blue box: PPE **1** (all samples, 5 μM, λ_{max}abs 428 nm). Green box: **1** in the presence of different metals (metal concentration: 0.4 mM). Red box: **1**·cofactor (cofactor concentration: 5 μM). Boxed data are controls. The picture shows the fluorescence of different samples under a hand-held UV light (λ_{max} 365 nm). Metal cations are in the +2 oxidation state, with the exception of Fe, which is Fe^{3+}.

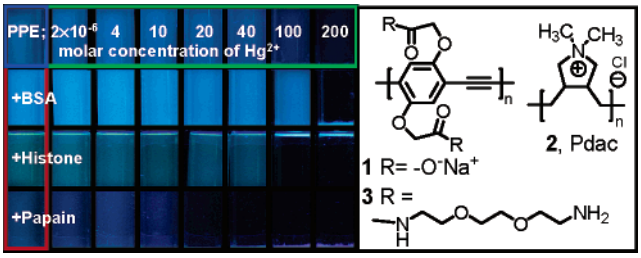

Figure 2. Left: addition of increasing concentrations of Hg^{2+} (mol/L) to **1** (5 μM) and its protein and polymer complexes (5 μM protein). Green box: PPE **1** + increasing concentration of Hg^{2+}. Red box: **1**·protein complexes. Blue box: PPE **1** alone. All boxed data are controls. The picture shows the fluorescence of the samples under a hand-held UV light (λ_{max} 365 nm). Right: chemical structures of **1**−**3**.

The cofactors change the response of **1** to a panel of metal cations (Figure 1).[13] In some cases (see Supporting Information), the high-energy part of the emission is more strongly quenched upon addition of the metal ions; this is probably due to concomitant metal-induced aggregation. The complex of **1**·papain gave agglutination with Hg^{2+}. Encouraged by these results, we investigated the lowest concentration of Hg^{2+} that led to optical changes of the **1**·cofactor complexes; **1**·BSA, **1**·histone, and **1**·papain were exposed to solutions containing diminishing concentrations of Hg^{2+} ions in water (Figure 2). PPE **1** and **1**·BSA are not very sensitive toward Hg^{2+} ions but show visible quenching and/or precipitation at a concentration of 0.1−0.2 mM Hg^{2+}. However, the **1**·papain complex is already agglutinated in the presence of 20 μM Hg^{2+} (Figures 2, 3). Although this is not sufficient to detect Hg^{2+} in environmental samples, it is as sensitive as most specifically designed, low molecular weight, mercury-detecting fluorophores.[14] How selective is this agglutination assay? Figure 4 shows the results of three experiments. Vial A contains the **1**·papain complex. Vial B contains the **1**·papain complex in the presence of a mixture of all tested metal cations, and vial C contains **1**·papain in the presence of nine metal cations

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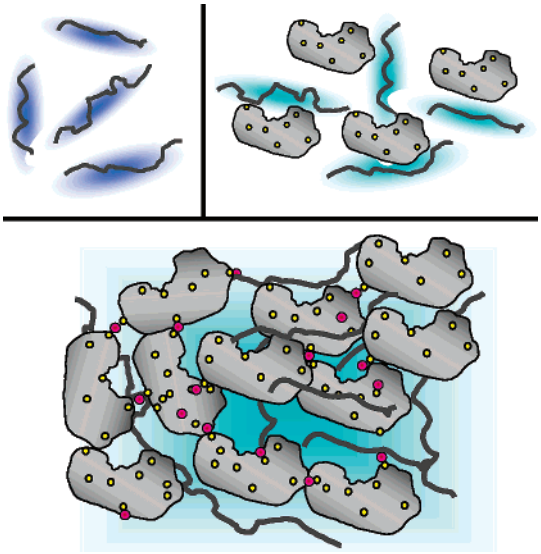

Figure 3. Qualitative interpretation of the Hg^{2+}-induced agglutination of the **1**·papain complex. Top left: **1** alone. Top right: electrostatic complex from **1**·papain. Bottom: the addition of Hg^{2+} to **1**·papain leads to its precipitation by cross-linking of the papain molecules through Hg^{2+}.

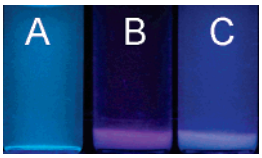

Figure 4. (A) PPE **1**·papain complex (**1**, 5 μM; papain, 5 μM). (B) All 10 metals added to **1**·papain complex (each metal: 0.4 mM). (C) Same without Hg ions. Picture shows fluorescence and was taken under a hand-held UV light (excitation wavelength of 365 nm).

but without Hg^{2+}. The fluorescence of the vials B and C is different, demonstrating that Hg^{2+} ions are *specifically* detected.

Why is the detection of mercury by **1**·papain more efficient than by either **1** or papain alone? Figure 3 shows the proposed mechanism of action for this agglutination assay. The complex **1**·papain forms as a mixture of oligomers. Positively charged papain molecules are held together by the negatively charged PPE chains. The complex **1**·papain *is more sensitive toward agglutination than its parts alone*. The complex will precipitate and leave the supernatant solution nonfluorescent because all of the chains of **1** are incorporated into the Hg^{2+}·**1**·papain agglutinate.

We have found a second system where the same principle can be exploited. The complex of **3**·myosin in phosphate buffer precipitates in 2−5 h. If the test solution is ≥0.1 mM of ATP, precipitation is not observed. In the case of myosin itself, only ≥1 mM adenosine triphosphate (ATP) prevents myosin from precipitation in phosphate buffer; ATP induces a large conformational change in myosin.[15] The **3**·myosin complex must show a conformation-dependent change in solubility, with the ATP-induced conformation of **3**·myosin being more soluble; this behavior could be used in an ATP sensor.

In conclusion, proteins can modulate the sensory properties of **1** or **3** through electrostatic complex formation. The detection of Hg^{2+} by **1**·papain is proof that specific properties of proteins strongly influence the emissive behavior of conjugated polymers. We will report on further expansion of the concept showing that conjugated polymer−protein complexes will be as powerful as their important and popular DNA hybrids.[16−18]

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Supporting Information Available: Experimental details, spectroscopic data, and Stern Volmer constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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