Sensory Polymers for Electron-Rich Analytes of Biological Interest

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Amplifying fluorescent polymers (AFPs) with conjugated structures are an expanding class of optical sensory materials.[1] Water-soluble ionic AFPs have been exploited with great interest for the detection of biological species, including sugars,[2] bacteria,[3] proteins,[4] and DNA.[5] These methods all rely on the high photoreduction potentials of the polymers or energy transfer. To complement these processes, we recently described a series of electron-deficient poly(*p*-phenylene ethynylene)s (PPE)[6] that behave as excited-state oxidants. The excellent response of these materials to indole suggested utility in biosensing applications. To realize this potential we herein report a water-soluble photooxidizing AFP (**A**), and its performance in photoinduced electron transfer (PET) based detection of amino acids, neurotransmitters, and proteins possessing electron-donating aromatic moieties in aqueous buffer.

Polymer **A** (Figure 1) is synthesized by the alkaline hydrolysis (aqueous LiOH in THF−H_2O) of its methyl ester (*M*n 17 g·mol^{-1} PDI = 1.96) (Scheme S1) and contains a [2.2.2] bicyclic ring system which prevents aggregation induced self-quenching that limits many water-soluble AFPs.[7] The polymer is purified after hydrolysis by dialysis against distilled water to remove oligomers and lower molecular weight fractions (MW cutoff: 10 000). The ester precursor is readily soluble in THF, chloroform, toluene, and xylene but insoluble in water. Polymer **A** is insoluble in the organic solvents mentioned, but it is completely soluble in water and DMF.

The absorption and fluorescence spectra of **A** were obtained in water at different pHs (pH 1−10) (Figure 1). The polymer features a strong absorption band which arises from a $\pi-\pi^*$ transition with strong blue-green fluorescence. The emission spectra are strongly pH-dependent whereas the concomitant changes in the absorption spectra of polymers are minor. In neutral and basic media, the absorption and emission spectra of **A** closely resemble the spectra of its methyl ester analogue in organic solvents ($\lambda_{max\ (abs)}$ = 405 nm; $\lambda_{max\ (emi)}$ = 432, 459 nm).[8] In contrast, under acidic conditions the fluorescence of **A** broadens to give a less intense new band that is red-shifted from fluorescence maxima at high pH.

The pH response of **A**'s fluorescence suggests that the polymer chains are completely soluble at high pH and that aggregated chains are present at low pH due to hydrophobic, $\pi-\pi$ interactions,[9] and possibly hydrogen bonding between COOH residues.[10] The fluorescence quantum yield of anionic **A** in water (pH 6−7) measured relative to quinine sulfate (Φ_F = 0.53 in 0.1 N H_2SO_4) was 0.20, which is relatively high compared with known ionic PPE derivatives in aqueous solutions (Φ_F = 0.03−0.10).

Because of its high ionization potential the neutral precursor polymer showed sensitivity opposite that of typical AFPs in organic solvents. Namely it is sensitive to molecules that are easily oxidized such as indole and aromatic amine compounds.[6] On the basis of these results, we anticipated that anionic **A** would have a high quenching efficiency with cationic analytes having electron-donating aromatic moieties in aqueous media. We report here **A**'s response to biologically relevant amino acids, neurotransmitters, and proteins (Table 1).

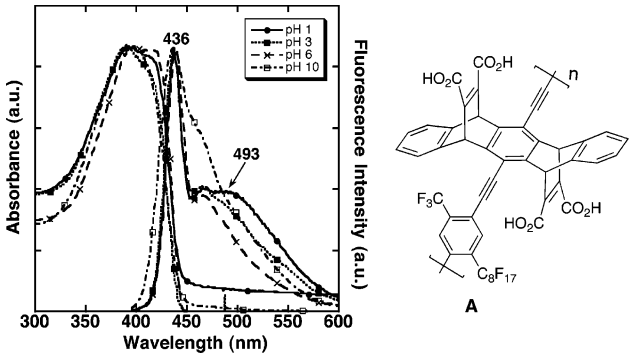

Figure 1. Normalized absorption and emission spectra of polymer **A** as a function of the solution pH. The optical density of polymer is 0.12−0.15 and the pH was adjusted with 0.1 N HCl or 0.1 N NaOH. Φ_F = 0.03 (pH 1), 0.05 (pH 3), 0.20 (pH 6), 0.22 (pH 10).

A feature common to all of the selected analytes (except alanine) is the presence of an aromatic system. The relative fluorescence quenching efficiency of **A** with given analytes was quantified in solution by determining the Stern−Volmer quenching constant (K_{SV}); $F^\circ/F = 1 + K_{SV}$[analyte] (Table 1).

Figure 2 shows the changes of emission intensity of **A** upon titration with tryptophan in phosphate buffer solution (100 mM, pH 7.4). The addition of tryptophan to solutions of **A** results in a large decrease in the fluorescence intensity, which is not accompanied by a new red-shifted emission peak. This result suggests that the fluorescence reduction is not the result of analyte induced conformational changes but solely through electron-transfer quenching.

The charge of amino acids is dependent on the pH of solution, and we expected that tryptophan (pI 5.89) would be negatively charged in the buffer solution (pH 7.4) and electrostatic repulsions should lower its ability to quench **A**. Nevertheless, anionic polymer **A** exhibited K_{SV} of 1400 M^{-1} with tryptophan, which is a 100-fold larger quenching efficiency relative to that observed for the neutral methyl ester precursor with indole in THF ($K_{SV} \sim 12$ M^{-1}).[6] All of the quenching we report herein is static in nature. The large quenching constants are not possible considering diffusional processes. For example, using the polymer's excited-state lifetime (0.32 ns) we calculate a bimolecular quenching rate constant of 3.6×10^{12} M^{-1} s^{-1} for tryptophan quenching in deionized water; this is the smallest quenching measured, but is orders of magnitude above the diffusion limit. To additionally confirm the static nature of quenching we determined the invariance of the polymer's lifetime as a function of tryptophan concentration (Figure S1). Tyrosine's low solubility at neutral pH necessitated K_{SV} determination in basic media and **A**'s K_{SV} for tyrosine (16 M^{-1}) was lower than that of tryptophan (80 M^{-1}) at pH 10. As is generally observed in ionic AFPs, the fluorescence quantum yield of anionic polymer **A** at pH = 6∼7 increased 4-fold to Φ_F = 0.80 in the presence of surfactant (neutral, 0.8% Triton X-100), suggesting hydrophobic interactions can exist between the polymer chains in aqueous media.[9] The addition of surfactant

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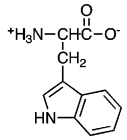
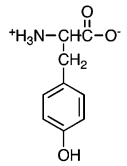
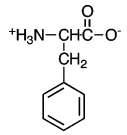
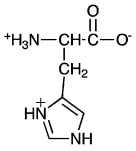
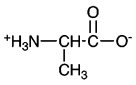
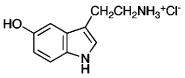
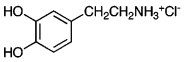
Table 1. Stern−Volmer Quenching Constants (M^{-1}) of A with Given Analytes in Aqueous Media at 25 °C

Analytes[a]		Phosphate buffer solution[b]	Deionized water (pH 6)
Tryptophan		1,400 ± 50	1,100 ± 30
Tyrosine		0	0
Phenylalanine		0	0
Histidine		0	0
Alanine		0	0
Serotonin		1.1 ± 0.2 x 10^5	4.4 ± 0.3 x 10^5
Dopamine		1.1 ± 0.1 x 10^4	3.0 ± 0.2 x 10^4
Lysozyme		6.1 ± 0.3 x 10^6	

[a] Results for L-amino acids are shown, but no difference was observed for D vs L. [b] 100 mM, pH 7.4.

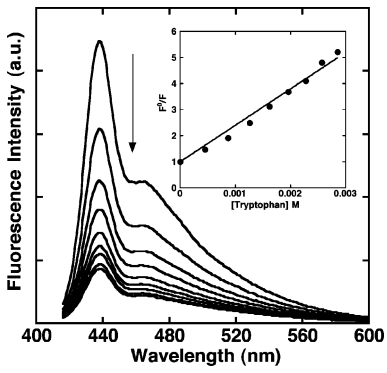


Figure 2. Changes in emission spectra (top) and S−V plot (inset) of polymer **A** upon addition of tryptophan in phosphate buffer (pH 7.4). [Trp] = 0 to 3 × 10^{-3} M (top to bottom).

to a polymer **A** solution with tryptophan in water decreased the quenching efficiency by 35-fold, which is attributed to a decreased interaction between the analyte and the conjugated polymer.

Aromatic amino acids lacking an easily oxidizable aromatic ring (phenylalanine or alanine) did not produce any fluorescence quenching responses with **A** at any pH even when added at high concentrations (up to 0.03 M). In addition, histidine, which has an isoelectric point higher than the pH of the buffer (pI 7.59), was expected to have ionic interactions as well as $\pi-\pi$ interactions. However histidine did not quench **A**'s fluorescence even at high concentrations (up to 0.04 M) at any pH. These results suggest that efficient electron transfer is the dominant factor in the fluorescence quenching mechanism of a photo-oxidizing **A** with amino acids with electron-donating aromatic units.

Fluorescence quenching responses of the electron-deficient anionic **A** were further investigated with the biologically important neurotransmitters,[11] serotonin and dopamine, in phosphate buffer solution (100 mM, pH 7.4). Serotonin showed a larger fluorescence quenching response with **A** than dopamine, as expected from its superior electron-donating abilities. The K_{SV} constants of dopamine and serotonin are much higher than those for tyrosine or tryptophan and can only be accounted for by an increased association between these positively charged neurotransmitters and the anionic polymer. Polymer **A** was also investigated as a molecular probe to detect water-exposed tryptophan and tyrosine residues in proteins. Lysozyme (chicken egg white, MW 14 307), which contains 6 tryptophans, showed a high fluorescence quenching response with polymer **A** (K_{SV} ~ 6 × 10^6 M^{-1}) in phosphate buffer solution (100 mM, pH 7.4). In contrast to previous studies of AFP quenching,[4b]

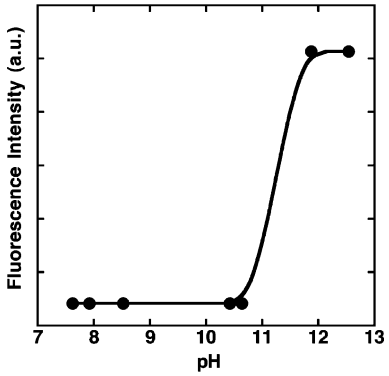


Figure 3. Change of fluorescence intensity of polymer **A** depending on pH of the mixture solution of polymer **A** and cytochrome c (excited at 435 nm).

continued addition of lysozyme to **A** did not show any saturation of quenching response. This result and the lack of any new spectroscopic features suggest that the fluorescence quenching between **A** and lysozyme is an electron-transfer process rather than modification of the polymer's self-quenching nature by electrostatic complexation as reported earlier.[4b]

On the basis of these results, we were interested to see if fluorescent conjugated polymers can provide broad-spectrum of protein detection as a function of charge. As an initial demonstration we undertook the determination of pI (isoelectric point) of cytochrome c (pI 10.65) by **A** (Figure 3). The fluorescence intensity of a solution composed of **A** and cytochrome c (1.25 μM) in water was monitored as a function of pH. At high pH (> pH 11) where both cytochrome c and **A** are both negatively charged, the association of polymer with protein is prevented and the fluorescence of **A** is not quenched. At lower pH, the charge polarity of protein is reversed, and fluorescence quenching of polymer **A** is observed. The interpolated fluorescence quenching response of polymer **A** on cytochrome c is comparable to the known pI of cytochrome c (Figure 3). This suggests a simple titration method for determining the pIs of proteins and such a scheme can be part of a protocol for rapidly identifying proteins.

In summary, a new water-soluble photooxidizing AFP has been synthesized and characterized. This work has shown that a water-soluble photooxidizing AFP could serve as a PET-based sensor for amino acids, neurotransmitters and proteins with electron-donating aromatic compounds in aqueous media. The results show that the quenching efficiency is strongly dependent on electrostatic interaction of the anionic polymer and quencher as well as hydrophobic and electron-transfer interactions between the polymer chains and quencher.

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Supporting Information Available: Text giving the synthesis and characterization of polymer and determination of Stern−Volmer quenching constants a scheme showing the synthesis of polymer **A** and a figure showing a plot of the Stern−Volmer constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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