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Amino Acid-Functionalized Polyfluorene as a Water-Soluble Hg^{2+} Chemosensor with High Solubility and High Photoluminescence Quantum Yield

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Water-soluble conjugated polyelectrolytes (CPEs) are conjugated polymers functionalized with multiple ionic groups.[1] CPEs combine optoelectronic properties of traditional conjugated polymers with the aqueous solubility and ionic nature of polyelectrolytes. CPEs can also be used to detect opposite ionic species in water solutions as fluorescence sensors. Especially they provide a useful platform for the development of highly sensitive fluorescence-based sensors for biomolecules, such as proteins, DNA, carbohydrates, and enzymes.[2] Most CPE sensors rely on a dramatic decrease in their inherent fluorescence intensity upon binding of an analyte.[3] Two well-known processes are electron transfer and energy transfer between CPEs and a quenching species.[3b−e] The other process for those nonquenching analytes is to indirectly cause fluorescence quenching by inducing aggregation of the fluorescent CPEs via electrostatic or hydrophobic interactions.[3f,g] For the analyte-induced aggregation, extremely large quenching responses of many CPE-based fluorescent sensors can be observed due to the enhanced exciton transport properties of a conjugated polymer by increasing the number of accessible exciton migration pathways in the aggregation states. Anyway, CPE itself should be highly emissive and soluble in aqueous solutions without any aggregation.

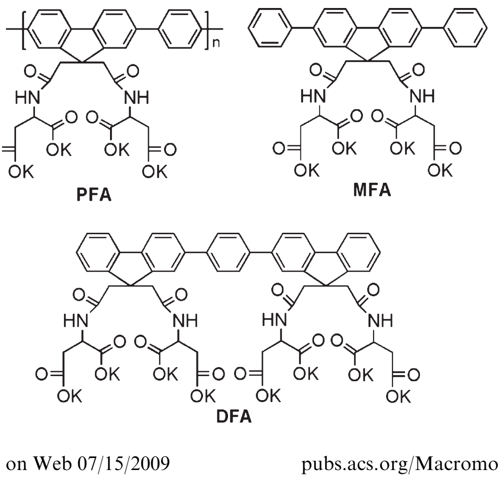
Water-soluble CPEs based on many kinds of conjugated polymers, such as polythiophenes, poly(*p*-phenylene vinylene)s, poly(*p*-phenylene)s, poly(phenylene ethylene)s, and polyfluorenes, have been developed.[4] Compared to their oil-soluble analogues, most reported water-soluble CPEs exhibit rather low solubility (typically <10 mg/mL) and low fluorescence quantum yield (Φ_{PL}) in water,[5] which are both due to the strong aggregation tendency of their hydrophobic conjugated backbones in water. These two drawbacks of CPEs greatly limit their real application as fluorescence CPE sensors. Many efforts have been devoted to overcome these drawbacks.[5] Ma et al. have obtained highly water-soluble (100 mg/mL) polyfluorene with bright fluorescence ($\Phi_{PL} \sim 44\%$) through introducing high charge density to side chains of polymer and adopting relative short side chain length.[5a] However, its Φ_{PL} is still not as high as those of typical polyfluorenes in organic solvents.[6] Bo et al. have reported a second-generation acidic dendronized polyfluorene with an extremely high Φ_{PL} (94%) due to the "site isolation" effect by the branched side chains.[5b] However, the presence of a lot of hydrophobic dendrons will obviously decrease its solubility in water, and the synthesis and purification of polyfluorenes containing the second-generation dendron is really tedious. Thus, a simple and reasonable way to achieve CPEs with both high fluorescence and high water solubility is to combine the above two methods.

Here, we report a novel water-soluble polyfluorene (PFA, Chart 1) with two anionic L-aspartic acids in its side chain of every repeat fluorene unit. First, the amino acid-functionalized side chain of PFA can serve as a good water solubilizer due to the high charge density. Secondly, the branched side chain will reduce the aggregation of polyfluorene and improve its Φ_{PL}. The last, but not least, polyamides have been reported as receptors for metal ions, especially for mercury ion recently,[7] so PFA is expected to coordinate efficiently with Hg^{2+} ion in aqueous solution. Although Hg^{2+} is a well-know highly toxic pollutant, only a few sensors have been reported to selectively detect Hg^{2+} in aqueous solutions.[8] It will be more desirable if PFA can be used as a fluorescence sensor for Hg^{2+}.

The water-soluble polymer PFA was obtained from Suzuki coupling reactions (Scheme S1 in Supporting Information (SI)). Perhaps due to the basic reaction condition, the carboxylic esters of monomers were hydrolysised completely during the reaction, which is confirmed by its ^1H NMR spectrum. PFA exhibits an absorption peak at 365 nm and an emission peak at 409 nm, similar as common fluorene−phenylene-based polymers in THF.[6] The water solubility of PFA (100 mg/mL) is already as high as the best reported value for CPEs, and its quantum yield (89%) is also comparable to that of typical polyfluorene in organic solvents. Unlike most water-soluble carboxylic acid-substituted conjugated polymers, there is almost no change in its fluorescene spectrum until pH = 7. At the same time, the fluorescence spectrum is also rather stable in 10 mM NaCl solution (Figure S1 in SI). Thus, as a CPE with high water solubility and high fluorescence quantum yield at the same time, PFA is a good candidate for fluorescence sensor material in aqueous solution.

PFA shows excellent selectivity for Hg^{2+} ion over most alkaline-earth cations, first-row transition-metal ions, group 12 metal ions, and common heavy-metal ions. As illustrated in Figure 1, upon addition of excess metal ions (4 μM), i.e., Ca^{2+}, Sr^{2+}, Cd^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Fe^{3+}, Pb^{2+}, and Al^{3+}, no significant changes of the fluorescence spectra were observed. When 4 μM Hg^{2+} was added into in a 10 mM phosphate buffer solution at pH 6.2 of PFA, PL intensity decreased dramatically with a 35-fold fluorescence quenching (Figure 1). In contrast, the

Chart 1



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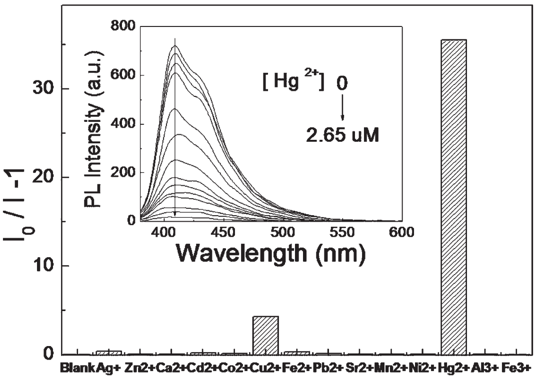

Figure 1. Fluorescence response profiles of PFA upon addition of 4 μM different cations in water at pH 6.2 with 10 mM $NaH_2PO_4-Na_2HPO_4$ buffer. [PFA] = 1 μM. Inset is fluorescence response of PFA to addition of Hg^{2+} in the buffer solution.

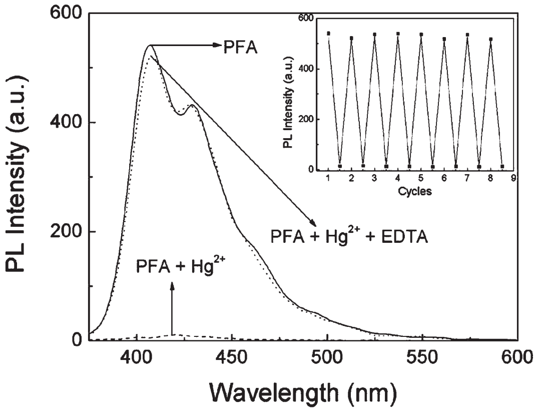

Figure 2. Fluorescence spectra of PFA with or without presence of Hg^{2+} and EDTA in water at pH 6.2 with 10 mM $NaH_2PO_4-Na_2HPO_4$ buffer. Inset is PL intensities at 410 nm of PFA upon cycling of adding Hg^{2+} and EDTA.

addition of 4 μM Cu^{2+} only induced a 4-fold fluorescence quenching, which is also a major interferent in most Hg^{2+} sensor systems. Anyway, the selectivity of PFA for Hg^{2+} over other metal ions is high (Figure S2 in SI). These results indicate that PFA is really a highly selective chemosensor material for Hg^{2+} ion in aqueous solution.

A detailed fluorescence titration of Hg^{2+} was conducted in pure buffer solution of PFA (1 μM). As we expected, the fluorescence intensity of the solution of PFA decreases upon addition of Hg^{2+} (inset in Figure 1). The Stern–Volmer plot (Figure S3 in SI) for PFA displayed a linear relationship within the Hg^{2+} concentration range employed (10 nM–2.65 μM). The corresponding K_{sv} value of PFA was determined to be 1.54 $\times 10^7$ M^{-1}. In the buffer solution, even the presence of an 10 nM of Hg^{2+} will lead to a 5% fluorescence quenching, indicating that Hg^{2+} could be detected at least down to 10 nM in the buffer solution, which is the EPA limit of Hg^{2+} ions in drinking water (10 nM).[8c] This limit of detection (LOD) is already comparable to those of previously reported conjugated polymer fluorometric Hg^{2+} sensors.[9]

It is important to indicate that the spectral sensing is reversible, so chelation experiments with EDTA were done in the buffer solution. The fluorescence was nearly recovered to original intensity of the PFA solution without Hg^{2+} (Figure 2). This off–on cycle can be repeated more than eight times without any observable deviations (inset in Figure 2). These results indicate that polymer PFA is a reversible chemosensor for Hg^{2+}.

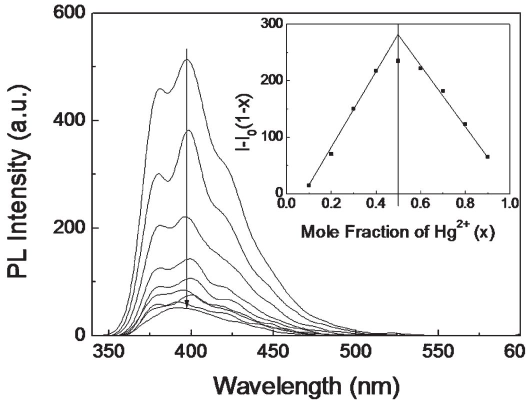

Figure 3. Fluorescence response of model compound DFA to addition of Hg^{2+} in water at pH 6.2 with 10 mM $NaH_2PO_4-Na_2HPO_4$ buffer. [DFA] = 5 μM. Inset is Job's plot of DFA in 10 mM phosphate buffer solution (pH = 6.2). The total concentration of DFA and Hg^{2+} ion is 10 μM.

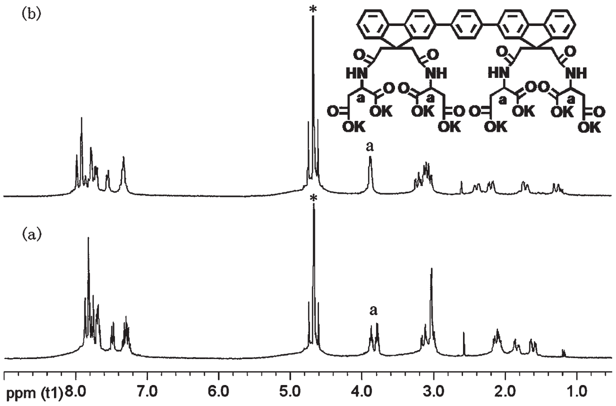

Figure 4. ^{1}H NMR spectra of free DFA (a) and upon addition of 3 equiv of Hg^{2+} ions (b) in D_2O.

To further clarify the chelation of Hg^{2+} with fluorene units containing anionic L-aspartic acid, model compounds MFA and DFA were synthesized. MFA contains two L-aspartic acid pendants in one fluorene unit. Its emission maximum is at 373 nm, and no clear fluorescence quenching can be observed upon the addition of Hg^{2+} (Figure S4 in SI). However, DFA behaves differently from MFA. Figure 3 shows its fluorescence response profile upon addition of Hg^{2+}. The emission maximum of DFA is at 398 nm. When Hg^{2+} (1 μM) was added into the buffer solution at pH 6.2 of DFA (5 μM), the fluorescence intensity was quenched to 26% of the original intensity without any change of its fluorescence maximum. The corresponding K_{sv} value of DFA was determined to be 1.14 $\times 10^6$ M^{-1} (Figure S5 in SI), 1 order of magnitude lower than that of PFA, which was consistent with the signal amplification ability of conjugated polymers due to facile energy migration along the polymer backbones.[10] At the same time, the absorption maximum of DFA is red-shifted from 331 to 341 nm gradually (Figure S6 in SI), suggesting the formation of a new nonemissive complex. Job's plot indicates that DFA forms a 1:1 complex with Hg^{2+}.[11] A possible explanation is the amide groups in the two neighboring fluorene units are need to take part in the coordination with Hg^{2+}, so only DFA exhibits fluorescence quenching upon addition of Hg^{2+}.

To further investigate the potential chelating behavior of DFA, ^{1}H NMR titration of DFA with Hg^{2+} was carried out in D_2O solution (Figure 4). When 3 equiv of Hg^{2+} ions is added, all aromatic peaks get broadened and shift downfield slightly, possibly due to a deshielding effect of the fluorene fluorophore. The aliphatic protons, around the amide groups, especially H_a, display the most obvious spectral change. DFA itself shows a set of two triple peaks of four H_a centered at 3.82 ppm, indicating their different chemical environment. Upon addition of Hg^{2+}, the H_a signal shift downfield to be a single peak (3.87 ppm), suggesting the chemical environment of all H_a turns almost same after coordination with Hg^{2+}. At the same time, the addition of Hg^{2+} have no effect on ^1H NMR of MFA. The ^1H NMR spectral behaviors imply that all the amide groups in the two neighboring fluorene units are in need to take part in the coordination with Hg^{2+} equally. The H_a signal of DFA keeps almost unchanged upon the addtion of Cu^{2+}, which also suggest Cu^{2+} can not coordinate efficiently with the amide groups as Hg^{2+}.

In summary, an anionic water-soluble polymer PFA has been synthesized by Suzuki coupling polymerization. PFA is a conjugated polyelectrolyte with an extremely high solubility in water (100 mg/mL) and high PL quantum yield (89%) comparable to those of typical polyfluorenes in organic solvents at the same time. PFA is also a highly sensitive, selective, and reversible sensory material for Hg^{2+} in buffer aqueous solution. Its LOD in high concentrated buffer solution can be as low as 10 nM, indicating that PFA compares favorably to previously conjugated-polymer-based Hg^{2+} sensors. Low cost and easy synthesis of this water-soluble fluorescence polymer and the robust aqueous detection condition make this system a good candidate for the development of applicable mercury sensors.

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Supporting Information Available: Synthetic and experimental details and spectroscopy data. This material is available free of charge via the Internet at http://pubs.acs.org.

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