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Fluorescence Quenching of a Partially Conjugated Polymer by Hemoglobin

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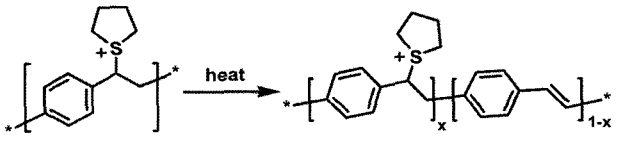
Introduction

Recently, the development of efficient sensors based on conjugated polymers has been central focus among many researchers.[1-3] Certain conjugated polymers undergo changes of properties such as absorption, emission, conductivity or redox potential upon environmental perturbations. If these properties can be manipulated by specific ligand-receptor interaction, the conjugated polymers can be used to detect biologically or chemically interesting target molecules. Accordingly, a variety of conjugated polymers have been extensively investigated as biological and chemical sensors.[4-10]

In order to make a water-soluble conjugated polymer, in general, tedious procedures are required since most synthetic methods developed for the synthesis of conjugated polymers are susceptible to the sidechain functionalties. Accordingly, protecting and deprotecting steps of functional groups which make the resulting polymers soluble in aqueous solvents are necessary. We thought if a commercially available poly (phenylene vinylene) (PPV) precursor, poly(*p*-xylene tetrahydrothiophenium chloride) **1**, shown in Scheme I can be transformed to a partially-conjugated polymer **2**, a water-soluble conjugated polymer could be readily obtained without further modification steps. In addition, the ionic conjugated polymer **2** is expected to be fluorescent due to the PPV moieties and the fluorescence of the polymer is expected to be affected by interaction with proteins. Herein, as part of our contribution toward conjugated polymer-based chemosensors,[11-13] we describe the fluorescence of the partially conjugated polymer **2** is quenched in the presence of hemoglobin.



Scheme I. Structures of a PPV precursor **1** and a partially conjugated polymer **2**.

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Experimental

Materials. Poly(*p*-xylene tetrahydrothiophenium chloride) (a PPV precursor) solution (2.5 wt% in water) was purchased from Aldrich. Hemoglobin, α-chymotripsin, bovine serum albumin (BSA) and papain were purchased from Sigma.

Preparation of a Partially Conjugated Polymer Solution. A 1 mL of poly(*p*-xylene tetrahydrothiophenium chloride) stock solution (0.25 wt% in H_2O) was diluted in HEPES buffer (5 mM, pH 8.0) to make a final concentration of 1.31 μM (monomer based). The resulting solution was heated at 100 °C for 3 min. Since prolonged heating results in aggregation of the polymer, heating was stopped before aggregation started to form.

Fluorescence Quenching Experiments. A typical experiment is as follows. To a 5 mL of HEPES buffer solution (5 mM, pH: 8.0) containing the partially conjugated polymer **2** obtained as described above was added 5 mg of hemoglobin. Fluorescence of the resulting solution was monitored using RF-5301PC Series Spectrofluorophotometer.

Micropatterned Fluorescence Images. A porous glass substrate was immersed in a solution containing the PPV precursor. The resulting film was heated at 100 °C for 1 min with a hotplate to transform PPV precursor to PPV. Hemoglobin was delivered to the PPV film using Gesim Nano-Plotter Model 2.0 (software NPC 16) array spotter.

Results and Discussion

At high temperature, the precursor polymer **1** is converted to the fully conjugated PPV.[14] The ionic PPV precursor polymer is soluble in aqueous solvent and becomes insoluble when transformed to fully conjugated PPV. If the ionic PPV precursor polymer **1** is converted to partially conjugated polymer **2**, it could be possible to obtain water-soluble PPV polymers. Accordingly, the commercially available precursor polymer **1** was diluted in HEPES buffer (5 mM, pH: 8.0), a slightly basic condition to promote elimination reaction. Heating of the solution resulted in a highly fluorescent PPV which was still soluble in aqueous solution due to the ionic tetrahydrothiophenium groups. Formation of the conjugated polymer was confirmed by observing increase in the absorption peak at ca. 320 nm. Since prolonged heating generates some polymeric aggregates, heating was controlled and stopped before the aggregates started to form. In this way, a water-soluble fluorescent conjugated polymer was readily

obtained without employing tedious sidechain modification steps routinely required for the preparation of the water-soluble conjugated polymers.

In order to investigate the effect of proteins on the fluorescence of the partially conjugated polymer **2**, the polymer **2** was exposed to commercially available proteins such as bovine serum albumin (BSA), α-chymotrypsin, and hemoglobin. Nonspecific interactions between ionic conjugated polymers and proteins have been reported.[9] We were curious whether the partially conjugated polymer **2** can interact with proteins and the fluorescence of the polymer could be altered by the proteins. Indeed, the fluorescence of the polymer **2** was affected by the presence of proteins. As displayed in Figure 1, all proteins tested were found to quench the fluorescence of the polymer. Interestingly, the fluorescence of the polymer was completely disappeared in the presence of hemoglobin. BSA and α-chymotrypsin are believed to change the conformation of the polymer **2** and quench the fluorescence by electrostatic interactions between surface ionic groups and the cationic moieties of the polymer **2**. On the other hand, hemoglobin has ion-containing heme moieties and the fluorescence quenching is presumably occurred by electron transfer from the PPV groups to heme moieties in the protein.[9]

Since the fluorescence quenching ability of hemoglobin is superior to other proteins, we further investigated the effect of hemoglobin concentration on the fluorescence quenching of the polymer **2**. As shown in Figure 2, the fluorescence intensity of the polymer decreased ca. 30% in the presence of hemoglobin at a concentration of 5 μg/mL and complete quenching was observed at a 200 μg/mL concentration.

The fluorescence quenching ability of hemoglobin was further demonstrated by observing this phenomenon by the naked eyes. As can be seen in Figure 3, the partially conjugated polymer **2** shows strong fluorescence under UV light (Figure 3(a)) due to the PPV moieties in the polymer chain. The presence of hemoglobin (5 μg/mL) resulted in the decreased fluorescence intensity as displayed in Figure 3(b).

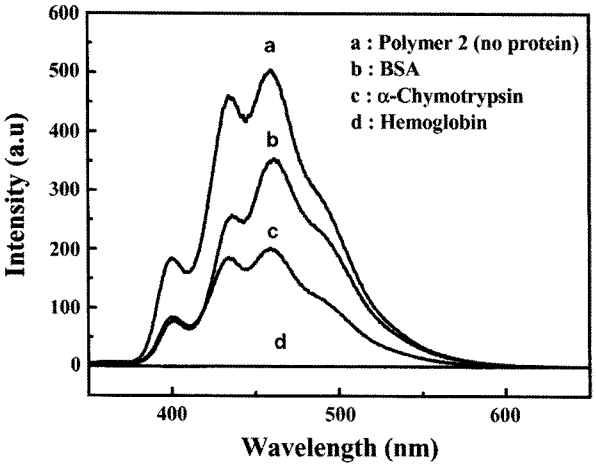

Figure 1. Fluorescence spectra of partially conjugated polymer solutions in the presence of proteins (1 mg/mL) in HEPES buffers (5 mM, pH: 8.0).

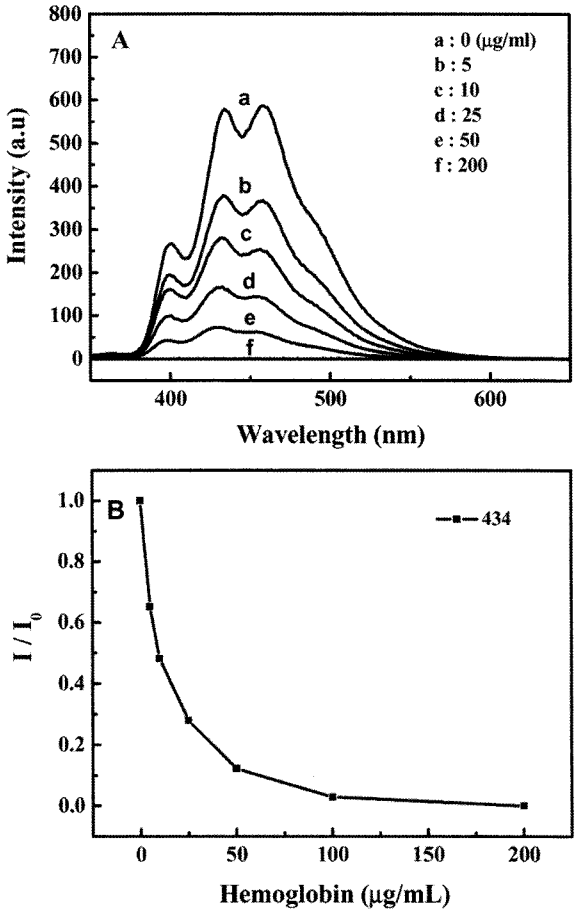

Figure 2. (A) Fluorescence spectroscopic monitoring of solutions containing the polymer **2** and various concentrations of hemoglobin. (B) Plots of I/I_0 as a function of hemoglobin concentration.

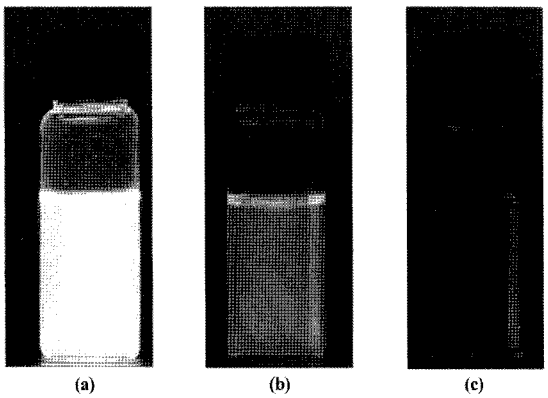

Figure 3. Photographs under UV light of solutions containing the partially conjugated polymer alone (a), in the presence of hemoglobin at a concentration of 0.005 mg/mL (b), and 0.5 mg/mL (c).

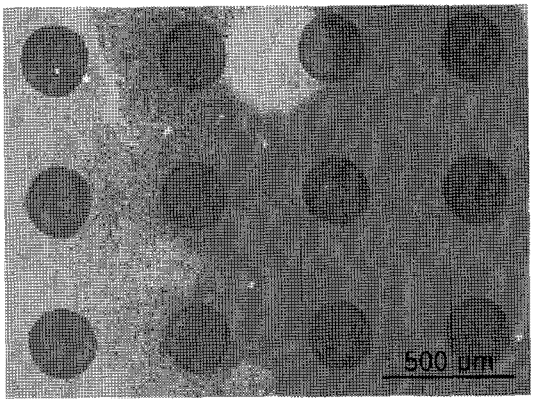

Figure 4. Patterned fluorescence images obtained by spotting hemoglobin solutions (1 mg/mL) onto the PPV film prepared as described in text.

The decreased fluorescence intensity of the polymer **2** in the presence of relatively low concentration of hemoglobin clearly supports hemoglobin is an excellent fluorescence quencher for the partially conjugated polymer **2**. Increase of hemoglobin concentration to 0.5 mg/mL results in the complete disappearance of the fluorescence (Figure 3(c)). The fluorescent quenching of the conjugated polymer by hemoglobin provides useful information on electron transfer properties between conjugated polymer and natural proteins.

We have been interested in the development of new strategies for patterned fluorescence images on solid substrates.[15-17] Hemoglobin was observed to be a very effective fluorescence quencher for the conjugated polymer **2**. We felt that if the fluorescence quenching could be achieved only in the limited areas on solid substrates, patterned fluorescence images could be obtained. In order to test this possibility, the PPV precursor polymer **1** was spincoated on a glass substrate. The resulting thin film was treated with a heatgun to transform the PPV precursor to PPV. Hemoglobin (1 mg/mL) was delivered to the PPV film using an array spotter. Patterned fluorescence images observed under a fluorescence microscopy is shown in Figure 4. The dark spots are the areas exposed to hemoglobin. The patterned images shown in Figure 4 demonstrates that a new strategy could be developed for the patterned fluorescence images based on the interaction between fluorescent conjugate polymer and protein quencher.

Conclusions

We have prepared a partially-conjugated water-soluble polymer by simply heating a solution of PPV precursor polymer. The partially conjugated polymer **2** obtained by this method was strongly fluorescent and the fluorescence was quenched by proteins such as bovine serum albumin, α-chymotrypsin, and hemoglobin. Hemoglobin was found to be superior to other proteins investigated in terms of quenching ability. The fluorescence quenching of the conjugated polymer **2** by hemoglobin is presumably due to the electron transfer from the polymer to heme moieties in the protein. Patterned fluorescence images were readily obtained by spotting hemoglobin on a PPV film by employing a modern array technology.

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