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Design and Synthesis of a New pH Sensitive Polymeric Sensor Using Fluorescence Resonance Energy Transfer

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In recent years, interest in stimuli-responsive materials is rapidly growing as a result of many potential applications in biotechnology such as target drug delivery and molecular sensors. In particular, molecular sensors capable of detecting environmental changes (temperature, pH, concentration of enzyme or ionic species, etc.) have been extensively sought for probing biological phenomena in living cells.[1−7] Intramolecular fluorescence resonance energy transfer (FRET), which is referred to as an energy transfer between donor and acceptor fluorophore introduced within a single molecule, offers a particularly facile way for monitoring a single molecular event such as molecular cleavage and conformational transition of a chain molecule and, therefore, provides a general platform to design a sensor molecule for detecting the variation in molecular environment because those molecular events usually occur reversibly or irreversibly at a specific condition. In earlier studies, the FRET has been introduced to investigate conformational dynamics of synthetic polymers and biopolymers by labeling fluorophores at specific sites in a molecule.[8−15] In such experiments, the variation in the FRET efficiency reflects the conformational change of a chain molecule because the efficiency is very sensitive to the distance between donor and acceptor fluorophore. In addition to these attempts for studying statics and dynamics of chain conformation, developing a novel synthetic FRET probe used for detecting the variations in environment or physiological phenomena has been extensively pursued. In particular, pH detection in aqueous media attracts much interest for medical, biological, and environmental applications. A prime example of such applications is provided by noninvasive optical detection for tumor cells, which is feasible by detecting a decrease in pH around tumor cells. One of the conceivable means for this purpose is to use a polymeric linker between donor and acceptor fluorophore in such a way that a pH-induced coil−globule transition of selected polymer linker gives rise to a drastic change in the donor−acceptor distance. As a result, the FRET shows on-and-off characteristics around a transition pH, where the coil−globule transition of polymer linker occurs.

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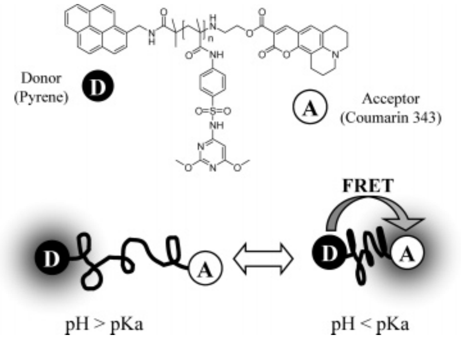

Figure 1. Chemical structure of P−PSDM−C and the schematic representation of the mechanism of FRET under change of pH.

This kind of "alarm"-type FRET probe is a convenient tool not only for an optical signaling of a diseased state such as the presence of tumor tissue but also for being an alarm for a hazard threshold in environmental applications. Some essential prerequisites should be satisfied for designing such an "alarm"-type pH sensor based on polymer linker: (1) the pH-induced conformational change from the expanded "coil" state to the collapsed "globule" state should be abrupt enough to show a typical two-state transition; (2) The molecular weight of a polymer linker should be optimally chosen such that the change in the donor−acceptor distance associated with the coil−globule transition induces well-defined on-and-off behavior with respect to the FRET; (3) the pH value at the coil−globule transition should be appropriate for being an alarm for the specific matter being monitored; and (4) the shift in the emission or excitation wavelength of acceptor fluorophore after the coil−globule transition of a linker should be large enough to be optically distinguished from the emission wavelength of donor fluorophore. The primary objective of this study is to design and synthesize a new polymeric pH sensor that shows a robust on-and-off characteristic in the FRET efficiency occurring at physiological pH.

The pH sensitive polymeric systems previously reported are mostly based on weak acid groups such as carboxylate, sulfonate, or sulfate,[16,17] and, therefore, these pH sensitive polymeric systems exhibit the pH induced conformational transition in the restricted range of pH only from 4.0 to 6.0 because of their intrinsic dissociation constants, pK_a.[18] Compared with those pH sensitive groups, a sulfonamide is one of the new class of pH sensitive materials, where its pK_a is easily controlled by changing an alkyl group of sulfonamide.[19,20] In this study, we choose one of the sulfonamides, sulfadimethoxine (SD), to introduce the pH sensitive property to the polymeric linker. The polymeric linker containing SD, as shown in Figure 1, was synthesized by the atom transfer radical polymerization (ATRP) which is one of the important controlled living radical polymerizations that allow well-defined polymers to be prepared.[21,22] It has been recognized that sulfonamide type monomers are not polymerized by the conventional ATRP method. Therefore, it is required to use a stronger ligand such as tris(2-dimethylaminoethyl)amine as the ATRP ligand for success of ATRP of sulfonamide containing monomers (see Supporting Information). Pyrene and coumarin 343 have been chosen as the FRET donor and acceptor, respectively. The most fascinating feature of this pair is that the presence of donor does not affect the relative fluorescence nor the wavelength maximum of the acceptor.[23] Pyrene was modified to be used as the ATRP initiator. When this modified pyrene is used as an initiator for ATRP, the resulting polymer has a pyrene moiety (FRET donor) to the one end of polymeric backbone (P−PSDM). By generating a hydroxyl group in the other end of polymeric backbone, the FRET acceptor, coumarin 343, is then conjugated with the hydroxyl group by esterification (see Supporting Information). Finally, as shown in Figure 1, both FRET donor and acceptor are then located at both ends of the pH sensitive polymeric backbone (P−PSDM−C). Because coumarin 343 is connected with the polymeric backbone through ester linkage, the ester linkage may be susceptible to hydrolysis in aqueous solution. However, it was realized that, under our experimental conditions, the coumarin 343 moiety is hardly dissociated from the polymeric backbone in aqueous solution even after 72 h, because the release experiment shows that the UV absorbance of the polymers in solution remains nearly unchanged for 72 h (see Supporting Information).

Figure 2A shows the emission spectra of P−PSDM−C as a function of pH, where all of spectra are obtained by irradiating the solution at 334 nm corresponding to the excitation wavelength of the FRET donor, pyrene. The shape of the overall emission spectra does not show any significant change as the pH value is decreased from 10.0 to 7.5. However, when the pH value is decreased below 7.0, it is observed that a new peak appears at 491 nm corresponding to the emission wavelength of coumarin 343. This is easily explained by considering the conformational change of polymeric linker (PSDM) with pH. Because the SD moiety is deprotonated and ionized at pH above the pK_a of SD, it is expected that P−PSDM−C is highly soluble at higher pH and, thus, the polymeric linker has an expanded chain conformation, whereas the P−PSDM−C becomes collapsed at pH below the pK_a of SD as a result of the hydrophobic nature of SD as it is protonated. Therefore, as the pH value is decreased below the pK_a of SD, the distance between pyrene (FRET donor) and coumarin 343 (FRET acceptor) becomes short enough to induce the effective FRET, as schematically shown in Figure 1. Because the FRET efficiency is inversely proportional to the sixth power of the distance between the FRET donor and the FRET acceptor,[8] the distance between pyrene and coumarin must be shortened for the effective FRET from pyrene to coumarin 343. When the intensity ratio of 491 nm (coumarin 343) to 378 nm

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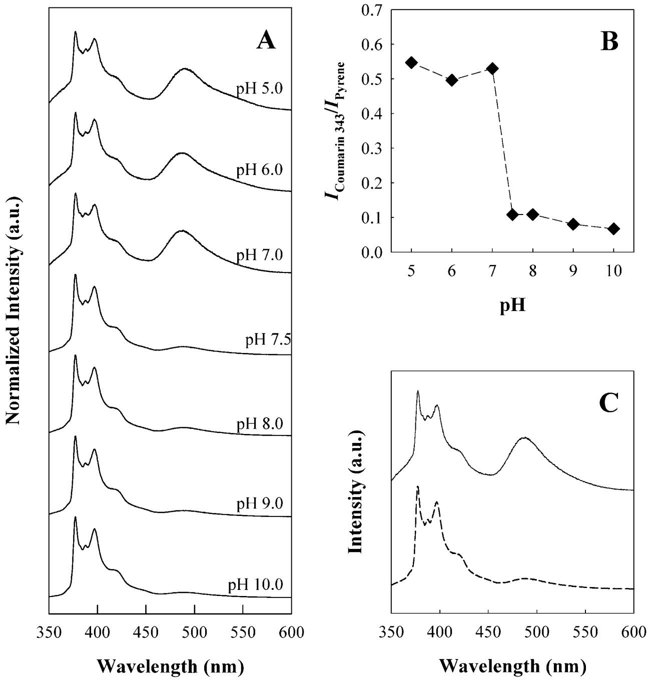

Figure 2. (A) Fluorescence emission spectra of P−PSDM−C at various pH values when the excitation wavelength of 334 nm is irradiated. The concentrations of P−PSDM−C are below 0.1 mg/mL for all cases. (B) Plot of intensity ratio of 491 nm (coumarin 343) to 378 nm (pyrene) as a function of pH. (C) Fluorescence emission spectrum of P−PSDM−C at pH 7.0 (solid line) and that of P−PSDM−C after increasing the pH from 7.0 to 8.0 (short dashed line).

(pyrene) is plotted against pH, as shown in Figure 2B, it is realized that the intensity ratio increases abruptly at around pH 7.0 as the pH decreases, indicating that the energy transfer from pyrene to coumarin 343 occurs below pH 7.0 with a sharp transition from coil to globule. Figure 2C demonstrates the reversibility of FRET. As the pH of the solution is raised from pH 7.0 to pH 8.0, the emission spectrum of pH 8.0 recovers its original spectrum, as shown in Figure 2C. This indicates that the polymeric linker becomes soluble at pH 8.0 and, thus, the chain is expanded, which leads to an increase in the distance between donor and acceptor and, therefore, a diminishing of the FRET efficiency. Another interesting feature noted from Figure 2 is that the further decrease below pH 7.0 does not significantly affect the FRET efficiency, once the polymeric linker is collapsed and the FRET from pyrene to coumarin 343 occurs at pH 7.0.

In summary, a new pH sensor was synthesized on the basis of a polymeric linker containing a sulfonamide group that undergoes the pH-induced coil−globule transition at physiological pH. The emission spectrum of this polymeric pH sensor demonstrates that the resonance energy transfer from pyrene (donor) to coumarin (acceptor) occurs effectively below pH 7.0. Because the FRET of P−PSDM−C responds to a relatively small change in pH, it is expected that this novel pH sensitive FRET system will be a promising candidate for investigating many biological phenomena.

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

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