Synthesis of a PEGylated Polymeric pH Sensor and Its pH Sensitivity by Fluorescence Resonance Energy Transfer

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ABSTRACT: A new pH-sensitive polymeric sensor with dispersion stability and biocompatibility is synthesized, and its pH sensitivity is examined on the basis of the fluorescence resonance energy transfer (FRET) efficiency. The polymeric pH sensor has a FRET donor and a FRET acceptor attached to both ends of a pH-sensitive polymeric linker and is also PEGylated to enhance the dispersion stability in aqueous media and biocompatibility. The pH sensor emits the blue color corresponding to the emission of the FRET donor at pHs higher than 7.6, but the pH sensor emits the green color corresponding to the emission of the FRET acceptor at pHs lower than 6.8 when both samples are irradiated at 330 nm, indicating that lowering the pH from 7.6 to 6.8 induces the FRET due to the conformational change of polymeric linker from coil to globule.

Introduction

Recently, stimuli-sensitive materials have been received much attention due to their wide variety of potential feasibility in biotechnology such as biomedical and biosensing applications. Particularly, developments of novel molecular sensors capable of detecting environmental changes (temperature, pH, concentration of enzyme or ionic species, etc.) have extensively been pursued in medical, biological, and environmental applications, since they can be used as an important tool for being an alarm for a specific threshold.[1] One of the promising methods for sensing those variations at a molecular level would be the fluorescence resonance energy transfer (FRET), where FRET is generally referred to as an energy transfer between fluorescent donor and acceptor.[2] Since the efficiency of FRET is very sensitive to the distance between FRET donor and acceptor, FRET has been traditionally used for monitoring a single molecular event such as the conformational transition of macromolecules by labeling fluorophores at specific sites.[2,3] Since the molecular event usually occurs under a specific condition, FRET possibly provides a general platform to design a stimuli-sensitive molecular sensor by combining the variation in molecular environment with the conformational change. As one of conceivable means for this purpose, an alarm-type FRET probe was previously reported based on the concept of the coil−globule transition of a pH-sensitive polymeric linker with FRET donor and acceptor attached to both chain ends of the polymeric linker.[1h] The polymeric linker showed drastically the pH-induced conformation change from the expanded coil state to the collapsed globule state, which resulted in an abrupt on-and-off feature in the FRET efficiency due to the change in the distance between FRET donor and acceptor with pH variation.

This newly conceptional FRET sensor has many powerful advantages. Since the stimuli-induced coil−globule transition is abrupt enough to show a typical two-state transition, the FRET sensor would be an ideal alarm-type sensor for detecting an important threshold in biorelated applications. Moreover, the emission wavelength of the FRET acceptor after induced by the FRET mechanism is largely different from that of the FRET donor, which makes it possible to be optically distinguishable between two different states. However, although the FRET sensor possibly offers a facile way for monitoring the specific matter being concerned, additional improvements are required to maximize its utilization in the area of biorelated applications. One of the inevitable needs is the dispersion stability of sensor molecule in aqueous media. For the purpose, an introduction of poly(ethylene glycol) (PEG) to the sensor molecule is considered in this study. Over past decades, PEG has received a particular interest, since PEG is proven to be one of the most preferred and biocompatible synthetic polymers for biorelated applications. Many researchers have reported numerous examples to support the importance of introduction of PEG to biological systems: improved solubility in aqueous media, nontoxicity, longevity in bloodstream, resistance to proteolysis, reduced immunogenicity and antigenicity, resistance to nucleases, ability to escape from reticuloendothelial system, reduced thrombogenicity, reduced protein and cell adherence.[4] Therefore, the introduction of hydrophilic PEG block, so-called PEGylation, to the FRET system is expected to enhance the dispersion stability (long-term stability) in aqueous media as well as the above-mentioned properties, which are essential for biological applications under various physiological conditions.

In our previous paper,[1h] we reported the design and synthesis of a new polymeric pH sensor that showed a robust on-and-off characteristic in the FRET efficiency occurring at physiological pH. However, the sensor may not be satisfactory for medical and biological applications, since it is not PEGylated. In this paper, we report the synthesis of a PEGylated polymeric pH sensor and its pH sensitivity on the basis of the FRET efficiency.

Experimental Section

Materials. All reagents were purchased from Sigma-Aldrich unless noted. Tetrahydrofuran (THF) (Daejung Chemicals & Metals) was dried over sodium and distilled under atmospheric pressure. 7-Acetoxy-4-bromomethyl coumarin (Tokyo Chemical Industry), concentrated HCl (Daejung Chemicals & Metals), methylene chloride (Daejung Chemicals & Metals), anhydrous magnesium sulfate (Daejung Chemicals & Metals), monomethoxy poly(ethylene glycol) (mPEG) (M_n = 2000, Sunbio Corp.), succinic anhydride (99+%), carbon tetrachloride (99.5%, Daejung Chemicals & Metals), diethyl ether (99.0%, Daejung Chemicals & Metals), 1,3-dicyclohexylcarbodiimide (DCC, 1.0 M solution in dichoromethane), Celite 521 (Celite), methanol (Daejung Chemicals & Metals), tris-(2-aminoethyl)amine (97%, Lancaster Chem.), formalin (Yakuri Pure Chemicals), formic acid (Yakuri Pure Chemicals), KOH (Duksan Pharmaceutical), sulfadimethoxine (SD) (Tokyo Chemical

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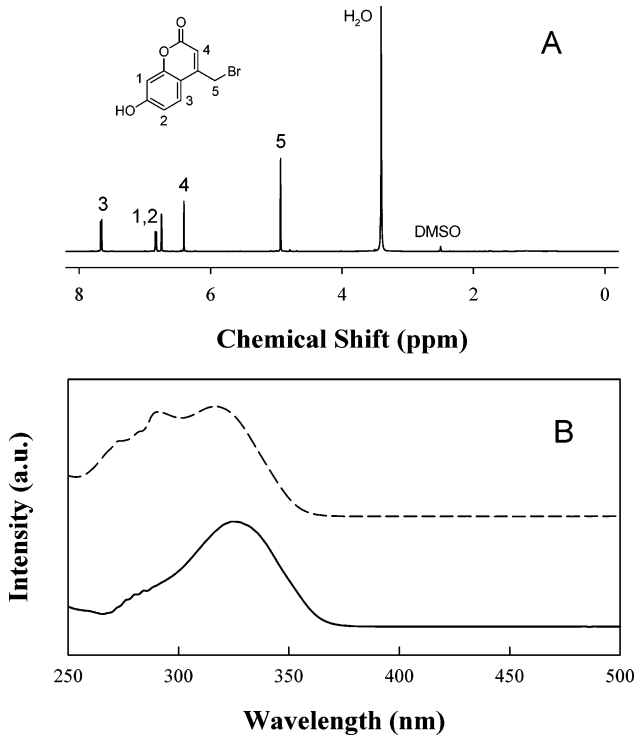


Figure 1. (A) ^{1}H NMR spectrum of **1** in DMSO-d_6. (B) UV−vis spectra of 7-acetoxy-4-bromomethylcoumarin (dashed line) and **1** (solid line) in THF.

Industry), NaOH (Duksan Pharmaceutical), acetone (Burdick & Jackson), methacryloyl chloride (97%), copper(I) bromide (99.999%), *N*,*N*-dimethylformamide anhydrous (DMF) (99.8%), methanol (99%, Daejung Chemicals & Metals), dimethyl sulfoxide (DMSO) (Tokyo Chemical Industry), triethylamine (99.5%), 2,2′-(ethylenedioxy)bis(ethylamine) (EDBEA) (98%), coumarin 343 (C343), anhydrous dimethyl sulfoxide (anhydrous DMSO) (99.9%), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (DEC) (98+%), and *N*-hydroxysuccinimide (NHS) (97%) were used as received.

Synthesis of 7-Hydroxy-4-bromomethylcoumarin (1). First, 7-acetoxy-4-bromomethylcoumarin (3.00 g, 1.01×10^{-2} mol) was added to a two-neck round-bottom flask, followed by adding THF (100 mL). Concentrated HCl (5 mL) was then slowly added. The solution was stirred at room temperature for 48 h, and the product was dissolved in methylene chloride and washed with distilled water three times. The organic phase was dried over anhydrous magnesium sulfate. After filtering and removal of solvents, the final product **1** was obtained as a solid. Yield: 82.9%. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of **1** and UV−vis spectra (in THF) of 7-acetoxy-4-bromomethylcoumarin and **1** are shown in Figure 1.

Synthesis of Carboxylated PEG (2). mPEG (5.00 g, 2.50×10^{-3} mol) was added to a two-neck round-bottom flask, and the flask was evacuated under 100 °C for 6 h to completely remove water. The flask was then cooled to room temperature and charged with N_2 gas before succinic anhydride (0.75 g, 7.50×10^{-3} mol) was added. The reaction mixture was allowed to stir at 130 °C for 6 h. The crude product was dissolved in carbon tetrachloride, and the solution was filtered to remove unreacted succinic anhydride. The solution was precipitated in cold diethyl ether for purification. After filtration, the precipitate was dried in a vacuum at 35 °C. The final product **2** was obtained as solid. Yield: 53.1%. The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **2** is shown in Figure 2.

Conjugation of 2 with 1. The carboxylated PEG **(2)** (3.68 g, 1.57×10^{-3}) was added to a two-neck round-bottom flask, and the flask was evacuated under 100 °C for 6 h to completely remove water. After the flask was then cooled to room temperature and charged with N_2 gas, purified THF (50 mL) was added. After **1** (1.2 g, 4.70×10^{-3}) was added, DCC (4.70 mL) was slowly added. The reaction mixture was stirred at 35 °C for 48 h. The crude

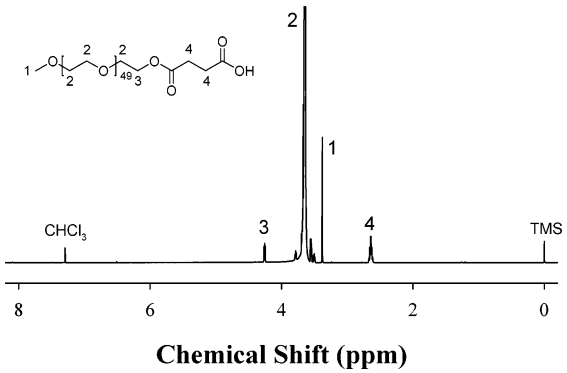


Figure 2. ^{1}H NMR spectrum of **2** in $CDCl_3$.

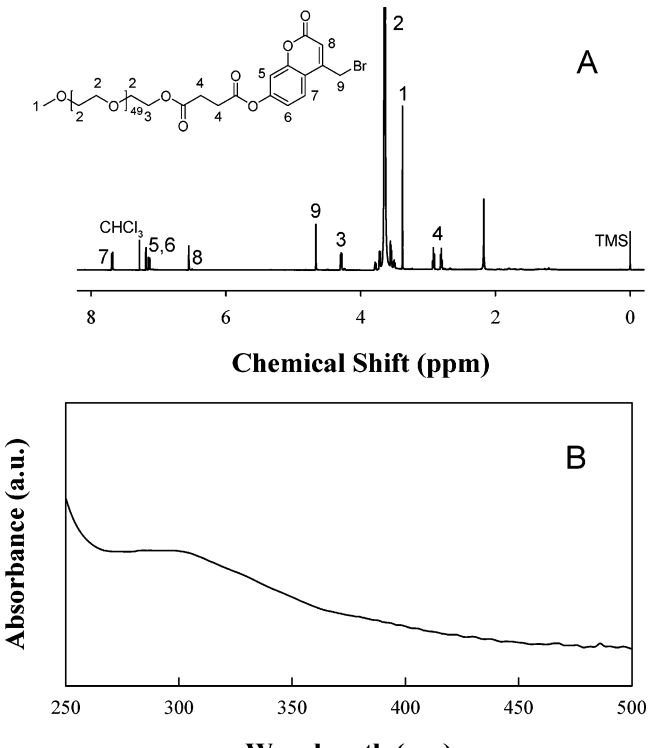


Figure 3. (A) ^{1}H NMR spectrum of **3** in $CDCl_3$. (B) UV−vis spectrum of **3** in pH 8.0.

product was filtered through the Celite column and dialyzed against methanol to remove unreacted reactants using a cellulose dialysis membrane (molecular weight cutoff: 1000, Spectrum Laboratories Inc.). For further purification, the dialysis against THF was additionally carried out. The solution was then precipitated in cold diethyl ether, and the final product **3** as a precipitate was dried under vacuum at 35 °C. Yield: 47.2%. The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) and UV−vis spectrum (in pH 8.0) of **3** are shown in Figure 3.

Synthesis of Tris(2-dimethylaminoethyl)amine. Tris(2-dimethylaminoethyl)amine (Me_6TREN) was prepared according to the previous report.[5] Tris(2-aminoethyl)amine (36.27 mL, 0.243 mol) was added to a three-neck round-bottom flask, and the flask was cooled to 0 °C. Concentrated HCl (59.8 mL) was slowly added to the flask, and 30 mL of distilled water was then slowly added. Formalin (170 mL) and formic acid (200 mL) were successively added. The flask was then placed in a PEG bath thermostated at 120 °C for 6 h. After all the volatile fractions were removed by vacuum distillation, the residue was treated with 400 mL of 10% NaOH aqueous solution. An oily layer was extracted by diethyl ether, and the ethereal extract was then dried over KOH. After removal of ether, the final product was obtained as colorless oil by vacuum distillation. Yield: 30.0%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of Me_6TREN is shown in Figure 4.

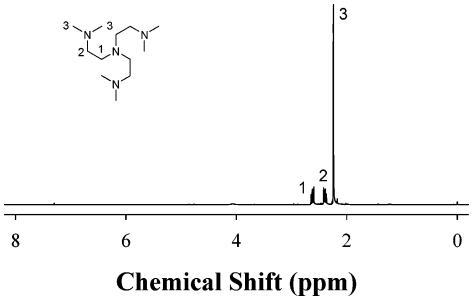


Figure 4. ^{1}H NMR spectrum of Me_6TREM in $CDCl_3$.

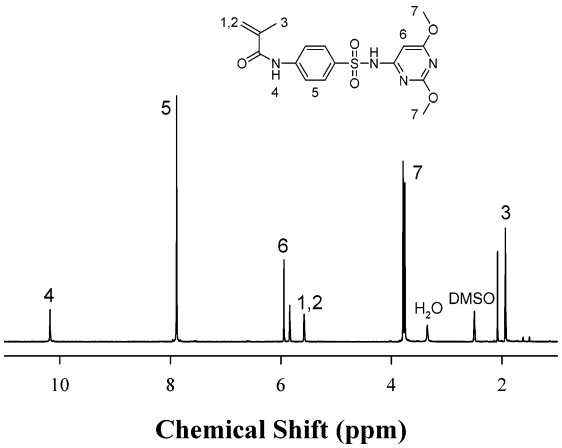


Figure 5. ^{1}H NMR spectrum of **4** in DMSO-d_6.

Synthesis of Sulfadimethoxine Containing Monomer 4. Monomer **4** containing a sulfadimethoxine moiety was synthesized according to the previous report.[1b] SD (30 g, 9.67 × 10^{-2} mol) was added to a three-neck round-bottom flask, and 200 mL of distilled water containing NaOH (3.87 g, 9.67 × 10^{-2} mol) and 200 mL of acetone were successively added to the flask. After the flask was cooled to 0 °C, methacryloyl chloride (9.45 mL, 9.67 × 10^{-2} mol) was slowly added to the flask. When the reaction flask was stirred at room temperature for 12 h, the product **4** was precipitated. The precipitated product was collected by filtration and washed with distilled water three times. The white product was then dried at room temperature for 24 h. Yield: 96.2%. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of **4** is shown in Figure 5.

Atom Transfer Radical Polymerization of 4 Using 3. Monomer **4** was polymerized by ATRP using **3** as a macroinitiator, producing the polymer **5**. DMF was degassed for removal of oxygen by three freeze-and-thaw cycles. Distilled water was degassed by boiling at 130 °C for 48 h and bubbling with N_2 gas for 12 h to completely remove dissolved oxygen. Monomer **4** (2 g, 5.29 × 10^{-3} mol) was put in a three-neck round-bottom flask, and the flask was degassed and backfilled with N_2 gas. DMF (5 mL) was added to the flask, and then 10 mL of an aqueous NaOH (0.211 g, 5.29 × 10^{-3} mol) solution was added. Me_6TREN (0.076 mL, 2.74 × 10^{-4} mol) was added to the flask via a microsyringe, and copper(I) bromide (0.039 g, 2.74 × 10^{-4} mol) was then added to the flask. A solution of **3** (0.707 g) in DMF (5 mL) was added to the flask via a syringe. The flask was then placed in a PEG bath thermostated at 35 °C for 2 h to allow ATRP. For purification of the product **5**, a solution of the crude product was precipitated in 1 N HCl solution. After filtration, the filtered product was immersed in a beaker with methanol and stirred at room temperature for 24 h to remove unreacted **4**. The final product was filtered and then dried in a vacuum at 30 °C. Yield: 20.0%; $M_{n,NMR}$: 12 000; PDI_{GPC}: 1.12. The ^{1}H NMR spectrum (300 MHz, DMSO-d_6) and UV−vis spectrum (in pH 8.0) of **5** are shown in Figure 6.

Introduction of Amine Group to 5. Polymer **5** (0.4 g) was placed in a three-neck round-bottom flask. DMSO (4 mL) was then added to the flask, and then triethylamine (0.0046 mL, 3.30 × 10^{-5} mol) and EDBEA (0.049 mL, 3.34 × 10^{-4} mol) were successively

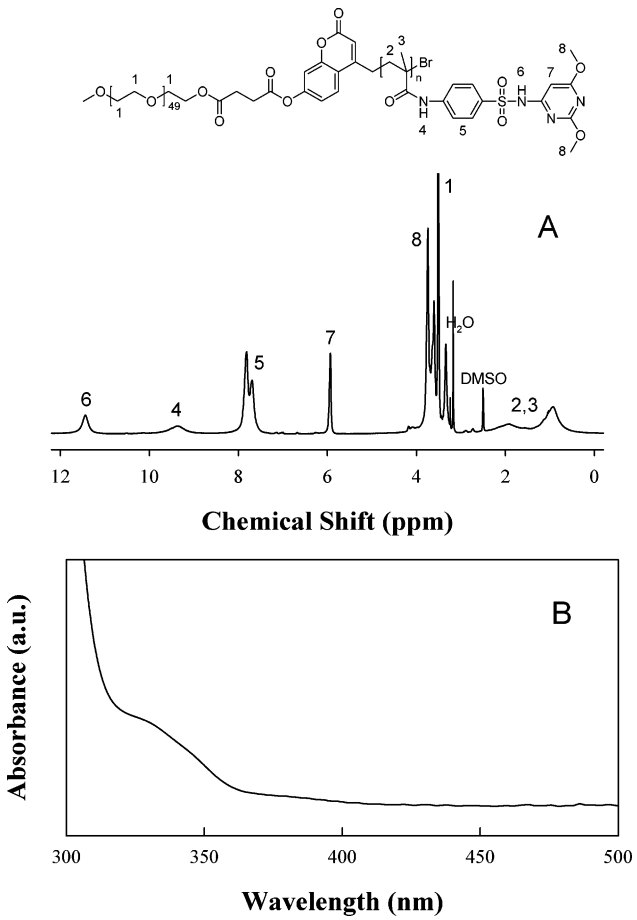


Figure 6. (A) ^{1}H NMR spectrum of **5** in DMSO-d_6. (B) UV−vis spectrum of **5** in pH 8.0.

added to the reaction flask via a microsyringe. Finally, distilled water (0.4 mL) was added to the flask via a microsyringe. The reaction flask was then placed in a PEG bath thermostated at 35 °C for 24 h. The reaction product **6** was purified by dialyzing against methanol for 48 h to completely remove unreacted reactants and byproducts using a cellulose dialysis membrane (molecular weight cutoff: 3500; Membrane Filtration Products, Inc.). The final solution was filtered, and the filtered product was dried in a vacuum at 30 °C. A ninhydrin test was performed to qualitatively verify the introduction of amine group (see Supporting Information). Yield: 84.5%. The UV−vis spectrum (in pH 8.0) of **6** is shown in Figure 7.

Conjugation of 6 with C343. Polymer **7** was synthesized by amidation between amine group of **6** and carboxylic group of C343. First, C343 (0.009 g, 3.15 × 10^{-5} mol), DEC (0.018 g, 9.39 × 10^{-5} mol), and NHS (0.018 g, 1.56 × 10^{-4} mol) were put together in a three-neck round-bottom flask, and the flask was degassed and backfilled with N_2 gas. Anhydrous DMSO (3 mL) was added to the flask, and the reaction was continued at 35 °C for 12 h under stirring. A mixture of **6** (0.25 g) and anhydrous DMSO (3 mL) was slowly added to the flask via a syringe. The flask was then placed in a PEG bath thermostated at 35 °C for 48 h. After filtration, the crude product **7** was purified by dialyzing against DMF for 24 h using cellulose dialysis membrane (molecular weight cutoff: 600−8000; Membrane Filtration Products, Inc.). Further dialysis against methanol was performed for 48 h to completely remove unreacted reactants and byproducts. The final solution was filtered, and the filtered product was dried in a vacuum at 30 °C. Yield: 67.2%.

Characterization. The structural analysis of materials used in this study was preformed by ^{1}H NMR (Avance DPX-300 or Avance 500, Bruker) and gel permeation chromatography (CTO-10A, Shimadzu) equipped with a refractive index detector (RDI-10A, Shimadzu) using DMF as an eluent, where the columns were

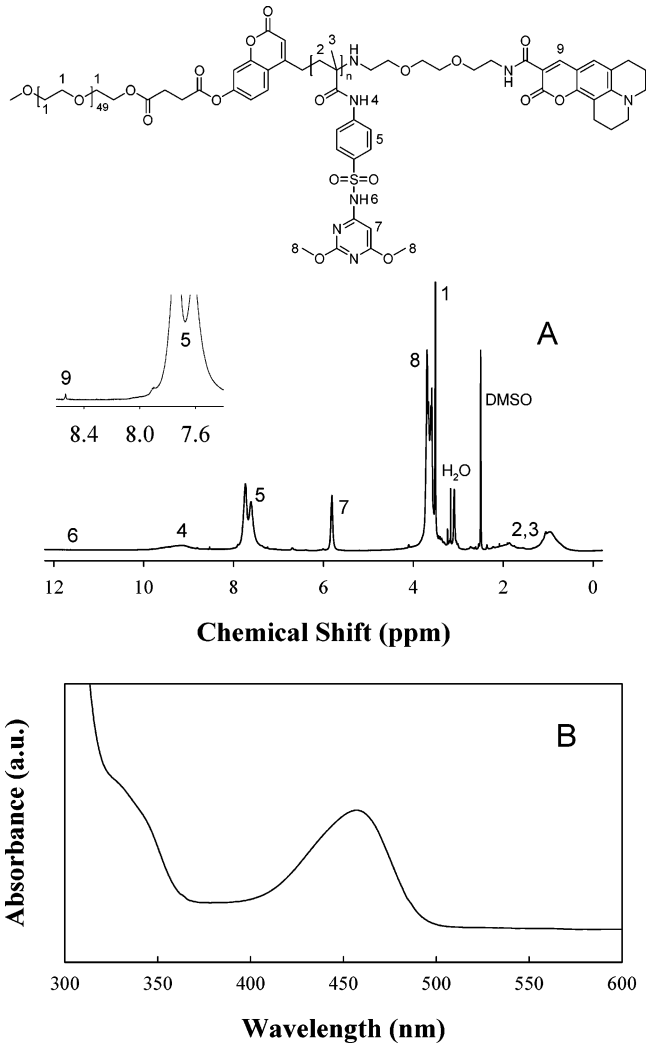


Figure 7. (A) ^{1}H NMR spectrum of **7** in DMSO-d_6. (B) UV−vis absorption spectrum of **7** at pH 8.0.

calibrated against three standard polystyrene samples (Shodex; $M_n = 1.31 \times 10^{-3}$, 1.39×10^{-4}, and 2.05×10^{-5}). UV−vis absorption spectra were obtained by a UV/vis spectrometer (HP 8452A, Hewlett-Packard). Fluorescence spectra were obtained from a fluorescence spectrometer (RF 5301, Shimadzu). The polymer solution was filtered using syringe filter (pore size: 0.20 μm, Minisart, Satorius) before the absorption and fluorescence spectra were taken. The florescence images were obtained by using a Xe lamp (Driel, 66208) with a monochromator (Driel).

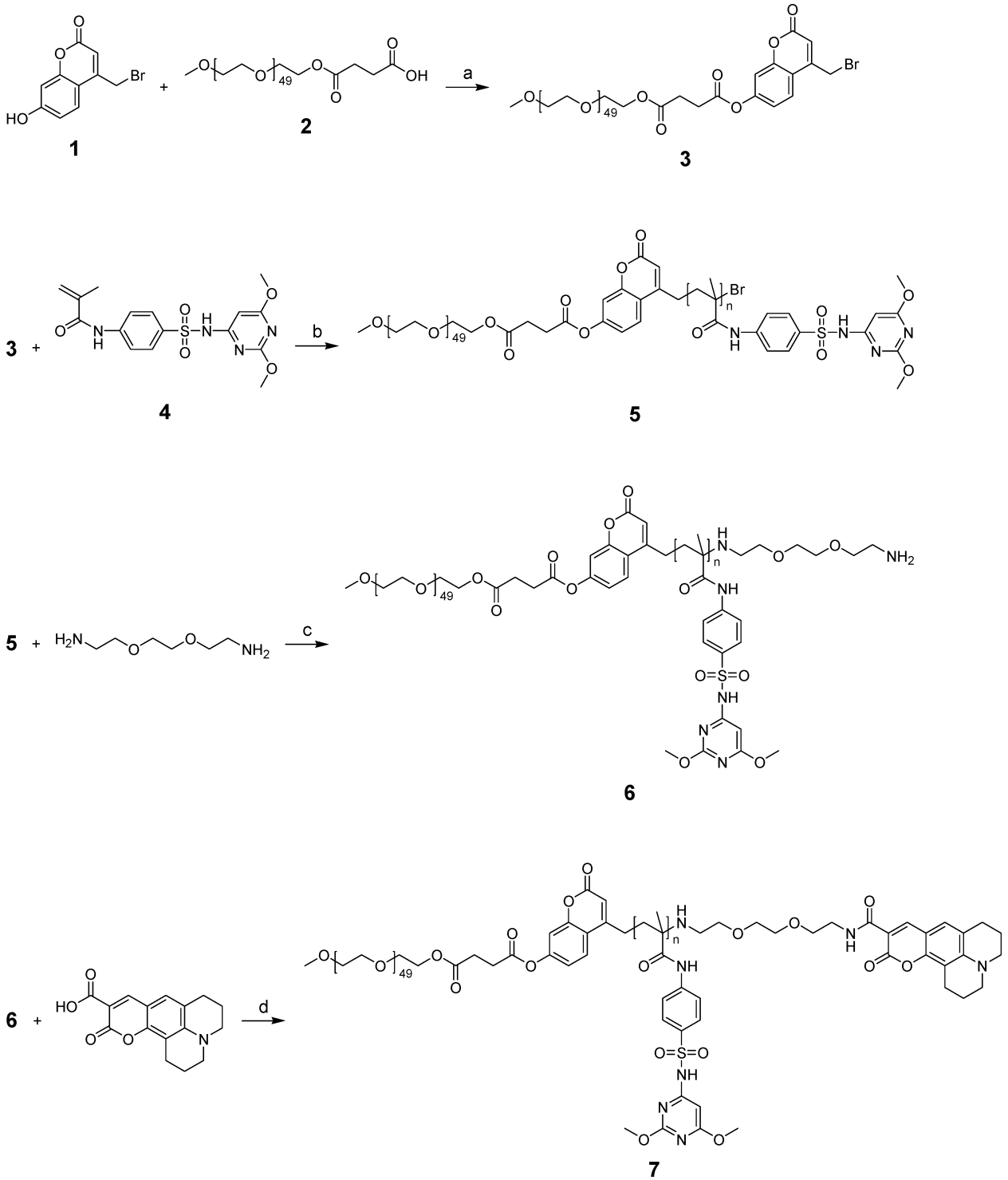
Results and Discussion

The overall synthetic route for the preparation of a PEGylated polymeric pH sensor is shown in Scheme 1. 7-Acetoxy-4-bromomethylcoumarin is modified under acidic conditions to yield 7-hydroxy-4-bromomethylcoumarin (**1**) which is used as a FRET donor, and the hydroxyl group of monomethoxy poly(ethylene glycol) is carboxylated by succinic anhydride to yield **2**. Compounds **1** and **2** are coupled via esterification using the carbodiimide coupling method, which results in **3**. It is noted that the compound **3** can be used not only as a macroinitiator for atom transfer radical polymerization (ATRP) but also as a FRET donor. A sulfonamide is selected to introduce the pH sensitive property to the polymeric linker because the sulfonamide has many advantages over conventional pH-sensitive materials. For example, its pK_a is easily controlled by changing an alkyl group of the sulfonamide, which enables the sulfonamide to be used in the broad range of pH.[6] As one of sulfonamide family, sulfadimethoxine (SD) is chosen since our previous study shows that the polymeric linker based on sulfadimethoxine changes its conformation in the range of physiological pH.[1h] The sulfadimethoxine monomer (SDM, **4**) is synthesized by reacting SD with methacryloyl chloride. The monomer **4** is then polymerized by ATRP using **3** as a macroinitiator. Consequently, polymer **5** has the FRET donor which is located between the PEG block and the one end of polymeric linker (PSDM). Here, it should be mentioned that a strong ligand such as Me_6TREN is needed for success of ATRP of **4**, since it has been recognized that sulfonamide-type monomers are not effectively polymerized by the conventional ATRP method (data not shown). For the introduction of C343 as a FRET acceptor to the other end of PSDM, the alkyl bromide at the polymer **5** is first reacted with EDBEA to generate **6** containing an amine group at the chain end, as shown in Scheme 1, and then C343 is conjugated with an amine group of **6** via the NHS activation and the carbodiimide coupling method to yield the final product **7**. Polymer **7** has the FRET donor at the one end and the FRET acceptor at the other end of polymeric linker PSDM and also has a PEGylated structure, where PEG is attached to the FRET donor. The absorption and emission spectra of **5** and C343 confirm the validity of the choice of **1** and C343 as a FRET donor and a FRET acceptor, respectively, as shown in Figure 8, where the maximum absorption spectra of both FRET donor and acceptor do not significantly overlap with each other, indicating that it is possible to excite the FRET donor alone at single wavelength. It also reveals that the emission spectrum of FRET donor overlaps sufficiently with the absorption spectrum of FRET acceptor. All of these features satisfy the condition of the overall integral factor for the successful FRET efficiency as reported by Förster.[2] It is also noted that the absorption spectrum of the FRET acceptor and the excitation spectrum of the FRET donor do not change in shape and intensity with pH variation (see Figure S1 in Supporting Information).

Scheme 2 illustrates the strategy for the sensing mechanism in this study. When pH is higher than pK_a of PSDM in **7**, PSDM has an extended conformation so that the FRET donor and the acceptor are thus apart from each other. When pH is lower than pK_a, PSDM collapses and therefore the FRET donor and the acceptor become closer, which results in the energy transfer of fluorescence as irradiated at the absorption wavelength of the FRET donor.

Figure 9 shows the variation of emission spectra of **7** as a function of pH, where all of the emission spectra are obtained by irradiating the solution at 330 nm corresponding to the absorption wavelength of the FRET donor. The shape of the overall emission spectra does not change significantly as the pH value is decreased from 8.0 to 7.6. However, when the pH value is decreased below 7.2, a new peak appears at 491 nm corresponding to the specific emission wavelength of the FRET acceptor, C343. When the intensity ratio of 491 nm to 380 nm is plotted as a function of pH, the intensity ratio increases steeply as the pH value is decreased below 7.6, as shown in Figure 10. This phenomenon is easily explained by considering the conformational change of PSDM with pH. Since the SD moiety in **7** is deprotonated and ionized at pH above the pK_a of SD, it is expected that PSDM is highly soluble in water at higher pH, and thus the polymeric linker PSDM has an expanded chain conformation, as shown in Scheme 2. However, PSDM becomes collapsed and has the globular structure at pH below the pK_a of SD due to the hydrophobic nature of SD, as it is protonated at lower pH. Consequently, the conformational change of PSDM

Scheme 1. Overall Synthetic Route[a]



[a] a, 1.0 M DCC, THF, 35 °C, 48 h; b, copper(I) bromide, Me_6TREN, NaOH/H_2O/DMF, 35 °C, 2 h; c, triethylamine, H_2O/DMSO, 35 °C, 24 h; d, DEC, NHS, anhydrous DSMO, 35 °C, 60 h.

from the coiled structure to the globular structure results in a decrease in the distance between FRET donor and acceptor, which enables to induce the effective FRET, as demonstrated in Scheme 2.

To demonstrate its feasibility to be an optically detectable sensor, the fluorescence images of two different states at pH 7.6 and 6.8 are obtained and compared with each other. When two solutions at pH 7.6 and 6.8 are excited with a single light source of 330 nm and the images are captured by a true-color digital camera without use of amplifiers and filters, it is observed that the solution at pH 6.8 emits the green color corresponding to the emission of the FRET acceptor while the solution at pH 7.6 emits the blue color corresponding to the emission of FRET donor, indicating that the FRET is effectively induced due to the conformational change of PSDM from coil to globule with pH variation, as shown in Figure 11. Since the FRET sensor in this study successfully demonstrates the color change from blue to green, this type of FRET sensor may exhibit any color change by choosing a proper pair of FRET donor and acceptor. Currently, several FRET systems showing multiccolor change have been studied in our laboratory, and the results will be reported in the future.

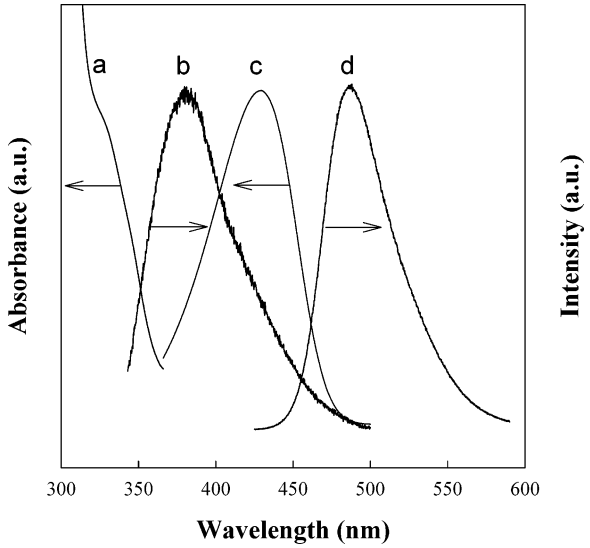
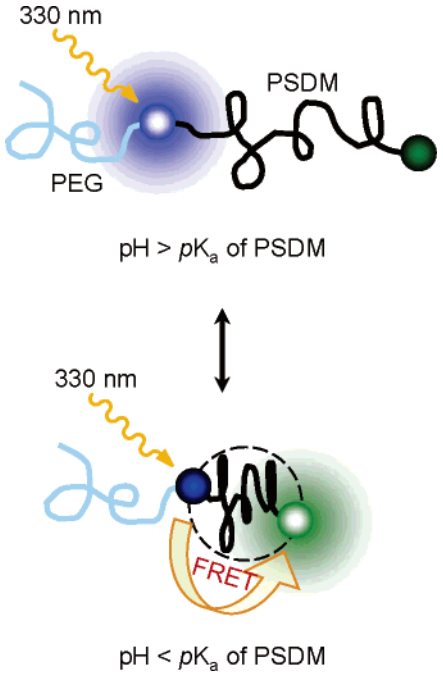


Figure 8. Normalized absorbance and emission spectra of **5** and C343 at pH 8.0: a and c are the absorbance spectrum of **5** and C343, respectively; b and d are the emission spectra of **5** (excited at 330 nm) and C343 (excited at 400 nm), respectively.

Scheme 2. Diagrammatic Representation of the Induction of FRET under Change of pH



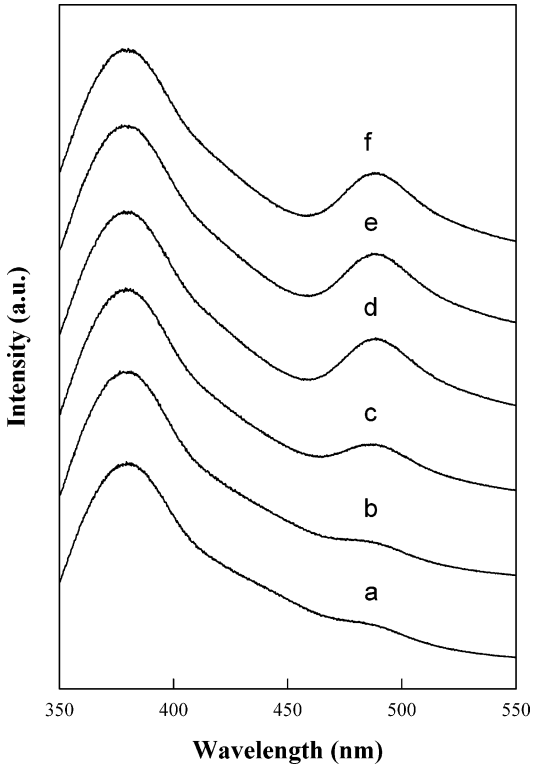


Figure 9. Fluorescence emission spectra of **7** at various pHs: a, 8.0; b, 7.6; c, 7.2; d, 6.8; e, 6.4; f, 6.0. The samples are irradiated at 330 nm, and the sample concentrations are below 2.0×10^{-3} g/L.

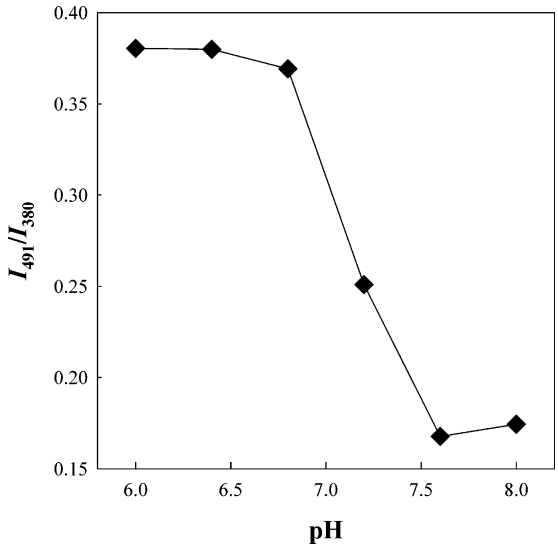


Figure 10. Plot of the ratio of the intensity at 491 nm (FRET acceptor) to the intensity at 380 nm (FRET donor) vs pH, when the pH sensor **7** is irradiated at 330 nm.

Conclusions

In this study, a new polymeric pH sensor with dispersion stability in aqueous media and biocompatibility is synthesized, and its FRET behavior is examined as a function of pH. The polymeric pH sensor consists of a pH-sensitive polymeric linker, PEG, a FRET donor, and a FRET acceptor. The polymeric linker containing the sulfonamide group undergoes the coil−globule transition under pH variation, and therefore the distance between the FRET donor and the FRET acceptor attached to both ends of the polymeric linker is changed with pH variation. When a solution of the polymeric pH sensor is irradiated at 330 nm, the solution at pH 7.6 emits a blue color corresponding to the emission of the FRET donor while the solution at pH 6.8 emits a green color corresponding to the emission of the FRET acceptor, indicating that the FRET is effectively induced due to the conformational change of the polymeric linker from coil to globule. Since this PEGylated FRET sensor responds to a relatively small change in pH with well-defined on-and-off behavior and also has dispersion stability in aqueous media, this novel pH-sensitive FRET system is expected to be a promising candidate for medical and biological applications.

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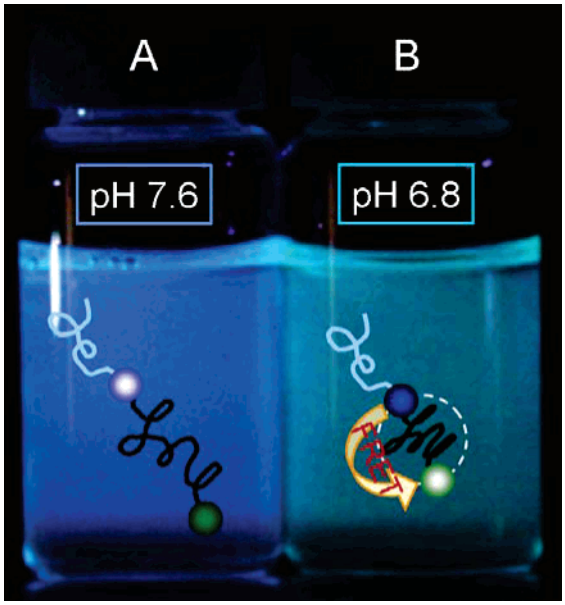

Figure 11. Fluorescence images of solutions containing **7** at (A) pH 7.6 and (B) pH 6.8 when the solutions are irradiated at 330 nm. Both solutions have the concentration of 2.0×10^{-2} g/L.

Supporting Information Available: Fluorescence emission spectra of **5** and UV−vis spectra of coumarin 343 (Figure S1), results of ninhydrin test (Figure S2), and UV−vis spectrum of **6**. This material is available free of charge via Internet at http://pubs.acs.org.

References and Notes

(1) (a) Yoshida, R.; Uchida, K.; Kaneko, Y.; Sakai, K.; Kikuchi, A.; Sakurai, Y.; Okano, T. *Nature (London)* **1975**, *374*, 240. (b) Park, S. Y.; Bae, Y. H. *Macromol. Rapid Commun.* **1999**, *20*, 269. (c) Suzuki, A.; Tanaka, T. *Nature (London)* **1990**, *346*, 345. (d) Kwon, I. C.; Bae, Y. H.; Kim, S. W. *Nature (London)* **1991**, *354*, 291. (e) Galaev, I. Y.; Mattiasson, B. *Trends Biotechnol.* **1999**, *17*, 335. (f) Roy, I.; Gupta, M. N. *Chem. Biol.* **2003**, *10*, 1161. (g) Gil, E. S.; Hudson, M. *Prog. Polym. Sci.* **2004**, *29*, 1173. (h) Hong, S. W.; Kim, K. H.; Huh, J.; Ahn, C.-H.; Jo, W. H. *Chem. Mater.* **2005**, *17*, 6213.

(2) (a) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Kluwer Academic/Plenum Publishers: New York, 1999. (b) van der Meer, B. W.; Cocker, G., III; Simon Chen, S.-Y. *Resonance Energy Transfer, Theory and Data*; VCH: New York, 1994.

(3) (a) Gopich, I. V.; Szabo, A. *J. Phys. Chem. B* **2003**, *107*, 5058. (b) Wang, D.; Geva, E. *J. Phys. Chem. B* **2005**, *109*, 1626. (c) Ratner, V.; Kahana, E.; Eichler, M.; Haas, E. *Bioconjugate Chem.* **2002**, *13*, 1163. (d) Rhoades, E.; Cohen, M.; Schuler, B.; Haran, G. *J. Am. Chem. Soc.* **2004**, *126*, 14686. (e) Ladokhin, A. S.; Haigler, H. T. *Biochemistry* **2005**, *44*, 3402. (f) Chen, M.; Ghiggino, K. P.; Mau, A. W. H.; Sasse, W. H. F.; Thang, S. H.; Wilson, G. J. *Macromolecules* **2005**, *38*, 3475.

(4) (a) Harris, J. M. *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Plenum Press: New York, 1992. (b) Harris, J. M.; Zalipsky, S. *Poly(ethylene glycol): Chemistry and Biological Applications*; American Chemical Society: Washington, DC, 1997.

(5) Ciampolini, M.; Nardi, N. *Inorg. Chem.* **1966**, *5*, 41.

(6) (a) Bell, P. H.; Roblin, R. O. *J. Am. Chem. Soc.* **1942**, *64*, 2905. (b) Foye, W. O. *Principles of Medicinal Chemistry*; Lea & Feiger: Philadelphia, 1989.

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