Fluorogenic Ion Sensing System Working in Water, Based on Stimulus-Responsive Copolymers Incorporating a Polarity-Sensitive Fluorophore

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Received July 24, 2007; Revised Manuscript Received October 15, 2007

ABSTRACT: We have developed fluorogenic (fluorescent off-on) ion sensors of a new type, based on stimulus-responsive synthetic copolymers incorporating a polarity-sensitive fluorophore. Upon binding a target ion in aqueous solution, these macromolecular fluorogenic sensors change their three-dimensional structure from an open form to a globular form at functional temperatures, and this induces a change of microenvironmental polarity around the polarity-sensitive fluorophore. The fluorophore transforms this environmental polarity change into a fluorescence signal. Our polymeric sensors were prepared by random copolymerization of comonomers selected from four types of comonomers. First, the role of each type of comonomer in the polymeric sensors was examined through the development of a fluorogenic H^+ sensor. Next, a fluorogenic K^+ ion sensor was developed in order to demonstrate the generality of this "buildup" design concept. Finally, a fluorogenic SO_4^{2-} sensor working in water was developed for the first time.

Introduction

Fluorometry has been widely used for microanalysis due to its high sensitivity and selectivity.[1] In order to apply fluorometry to nonfluorescent ions, various fluorogenic (fluorescent off-on) ion sensors have been developed.[2−4] These sensors show a significant fluorescence enhancement upon binding with a target ion. Most of the existing sensors utilize one of two fluorescence switching mechanisms,[3] i.e., control of a photoinduced electron-transfer process, and control of an intramolecular charge-transfer process involving the fluorophore. Nevertheless, it is still not easy to design fluorogenic ion sensors, particularly ones that will work in aqueous solution.[4]

A new type of fluorogenic ion sensor has recently been developed by combining polarity-sensitive fluorophores with stimulus-responsive macromolecules.[5,6] Upon binding a target ion, such a macromolecular fluorogenic sensor changes its three-dimensional structure at functional temperatures, resulting in a change of microenvironmental polarity around the polarity-sensitive fluorophore. The fluorophore transforms this environmental change into a fluorescence signal. Polypeptides and proteins have been used as the frameworks of such macromolecular sensors. For example, Imperiali et al. reported a fluorogenic Zn^{2+} sensor which consists of a Zn^{2+}-binding peptide and a dansyl fluorophore.[5a] A peptidyl fluorogenic Pb^{2+} sensor was also developed by Godwin et al.[5b] A similar strategy has been applied to protein-based sensors; e.g., Daunert et al. constructed a fluorogenic Ca^{2+} sensing system by means of site-directed mutagenesis of the Ca^{2+}-binding protein calmodulin and site-specific labeling with a fluorophore.[6a] One of the great advantages of these macromolecular fluorogenic sensors is their ability to function in water. However, rigorous fine-tuning of the peptide sequence is required in these sensors: not only the arrangement of the ion-binding domain, but also the position of labeling with the polarity-sensitive fluorophore is crucial, since the original function of the binding domain and the native conformation of the polypeptide and protein should be retained.

Recently, synthetic polymer-based macromolecular sensors, which utilize a similar fluorescence switching mechanism, have emerged. Schrader et al. reported dansyl-labeled fluorogenic polymers bearing bisphosphonate groups as receptors, which specifically recognize basic peptides and proteins, rich in arginine and lysine residues.[7] These polymers are constructed from more than one type of monomer unit, including a polarity-sensitive fluorescent monomer, by random polymerization of the comonomers. A series of fluorescent molecular thermometers reported by Uchiyama et al. was based on a similar fluorogenic mechanism, though the target was a physical property, temperature.[8] A polarity-sensitive benzofurazan fluorophore is randomly introduced into a temperature-responsive polymer, such as poly(*N*-isopropylacrylamide),[9] which changes its three-dimensional structure in water with increasing temperature. It is noteworthy that the range of temperature sensitivity and the excitation/emission wavelengths of these fluorescent thermometers are tunable: the former can be modified by replacing a comonomer with another having a different temperature response, and the latter can be changed by replacement of the comonomer containing a polarity-sensitive fluorophore with another having a fluorophore with different fluorescence properties. The extension of this design strategy to ion-responsive polymers[10] would allow us to construct a variety of tunable fluorogenic ion sensing systems that would work in water, and whose molecular design may be free from the need for rigorous sequence control of comonomers. Such ion sensors have been little studied so far.[11]

In this paper, we describe a fluorogenic ion sensing system based on a combination of an ion-responsive polymer, consisting of acrylamide derivatives, and a polarity-sensitive fluorophore, benzofurazan. Our fluorogenic ion sensors can be prepared by choosing suitable individual comonomers from among four types of comonomers, i.e., (i) an alkyl side chain for regulating functional temperature (**A1**, **A2**, and/or **A3** in Figure 1), (ii) a polarity-sensitive fluorophore (**B1**), (iii) a receptor for binding

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10.1021/ma071641i CCC: $37.00
Published on Web 11/21/2007

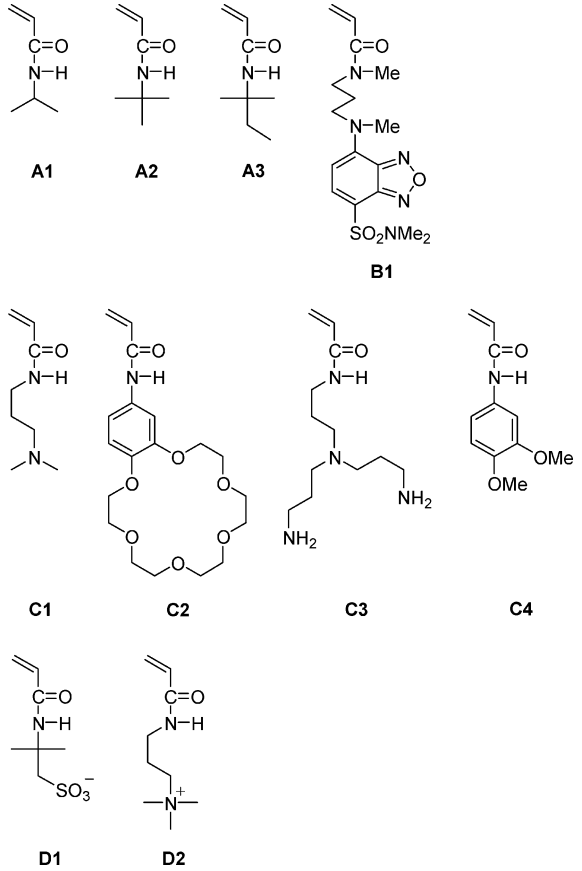

Figure 1. Chemical structures of acrylamide comonomers used in this study.

a target ion (**C1** for H^+, **C2** for K^+, or **C3** for SO_4^{2-}), and (iv) an ionic residue for controlling hydrophobicity/hydrophilicity and/or increasing the water solubility of the polymeric sensors (**D1** or **D2**). The sensors generated from these four types of comonomers can take an open form due to the formation of multiple hydrogen bonds between amide groups of the acrylamide linkage and solvent water when a target ion is absent. Upon binding a target ion, they can undergo three-dimensional conformational change to a globular form owing to hydrophobic attraction and/or electrostatic attraction, resulting in enhancement of the fluorescence signal. Moreover, we can easily tune their functions by changing a comonomer within one or more of the four types. The ability of these sensors to function in water opens up a wide range of applications, including bioimaging and environmental analysis. Herein, we first examine the role of each type of comonomer in our polymeric ion sensors through the development of a fluorogenic H^+ sensor. Second, a fluorogenic K^+ ion sensor is developed in order to demonstrate the generality of our design concept. Finally, a fluorogenic SO_4^{2-} sensor working in water is developed for the first time.

Results and Discussion

Fluorogenic H^+ Sensor Based on the Combination of a Stimulus-Responsive Polymer and a Polarity-Sensitive Fluorophore.

In order to demonstrate how the each of the four types of comonomer (**A**, **B**, **C**, and **D**, see Figure 1) affects the functions of the whole polymer, we selected a fluorogenic H^+ sensor as a representative sensor. Beginning with a homopolymer of *N*-isopropylacrylamide (**A1**), we sequentially added a comonomer from the each of the other types (**B**−**D**) or replaced one comonomer with another of the same type. Here, we used comonomer **B1** bearing a polarity-sensitive fluorophore, benzofurazan, and a *N*,*N*-dimethylaminopropylacrylamide (**C1**) unit bearing a basic tertiary amine as a proton receptor. Then, a set of copolymers **1**−**9** (Table 1) was synthesized by radical copolymerization. Ratios of each comonomer in the copolymers were fully optimized for the most effective function. Such ratios influence the function of the resultant copolymers to some extent, but in this paper, we focus only on the role of an added or a replaced comonomer, which is more crucial to fluorogenic ion sensing. Throughout this paper, we represent copolymers in terms of monomer ratios in the feed, though the real compositions of the copolymers were also determined (see Table 1). The fluorescence intensity and turbidity of **1**−**9** in water (under both acidic and basic conditions in the cases of copolymers **2**−**9**) were investigated at various temperatures to clarify the effects of each monomer unit on the H^+ sensing functionality.

Incorporating a Polarity-Sensitive Fluorophore, Benzofurazan, into a Temperature-Responsive Polymer, Poly(*N*-isopropylacrylamide) (Step I in Figure 2). A well-known temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PNIPAM), was first labeled with a small amount of benzofurazan fluorophore in the form of the comonomer **B1** (0.1 mol % in feed, this amount was unchanged throughout the study) to afford copolymer **1** ([**A1**/**B1**] = [100/0.1]). The fluorescence intensity of **1** in water increased sharply at 32 °C (Figure 2a), which was consistent with the previous report on fluorescent molecular thermometers.[8a] This event arises from the following characteristics of PNIPAM and comonomer **B1**; PNIPAM in water undergoes a phase transition above 32 °C, i.e., PNIPAM takes an open form below 32 °C due to hydration of the polymer chain, whereas it changes its three-dimensional structure to a globular form above 32 °C owing to hydrophobic attraction

Table 1. Physical Properties of the Synthesized Copolymers

copolymer	comonomer ratio in feed	yield (%)	component ratio in copolymer [a]	M_w [b]	M_n [c]	M_w/M_n
1	[**A1**/**B1**] = 100/0.1	83	100/0.088	1.26×10^5	4.48×10^4	2.81
2	[**A1**/**B1**/**C1**] = 90/0.1/10	82	91/0.043/9	9.76×10^4	3.02×10^4	3.23
3	[**A1**/**B1**/**C1**/**D1**] = 90/0.1/5/5	50	86/0.11/7/7	6.76×10^4	3.55×10^4	1.90
4	[**A1**/**B1**/**C1**/**D1**] = 88/0.1/5/7	64	86/0.086/6/8	7.33×10^4	3.73×10^4	1.97
5	[**A1**/**B1**/**C1**/**D2**] = 85/0.1/10/5	54	84/0.095/10/6	1.15×10^4	7.73×10^3	1.49
6	[**A2**/**B1**/**C1**/**D1**] = 57/0.1/20/23	68	49/0.12/22/29	9.68×10^4	4.38×10^4	2.21
7	[**A3**/**B1**/**C1**/**D1**] = 47/0.1/25/28	50	38/0.10/28/34	8.47×10^4	4.21×10^4	2.01
8	[**A2**/**B1**/**C1**/**D2**] = 65/0.1/30/5	13	61/0.10/30/9	9.77×10^3	6.74×10^3	1.45
9	[**A3**/**B1**/**C1**/**D2**] = 58/0.1/35/7	14	52/0.10/36/12	5.90×10^3	4.70×10^3	1.26
10	[**A2**/**B1**/**C2**/**D1**] = 68/0.1/12/20	74	62/0.11/14/24	7.18×10^4	3.87×10^4	1.86
11	[**A2**/**B1**/**C4**/**D1**] = 68/0.1/12/20	61	62/0.10/14/24	7.26×10^4	4.77×10^4	1.52
12	[**A2**/**B1**/**C3**] = 90/0.1/10	6.3	85/0.10/15	ND[d]	ND[d]	

[a] The order of components is the same as that in the "comonomer ratio in feed" column. [b] Weight-average molecular weight. [c] Number-average molecular weight. [d] Could not be determined by GPC.

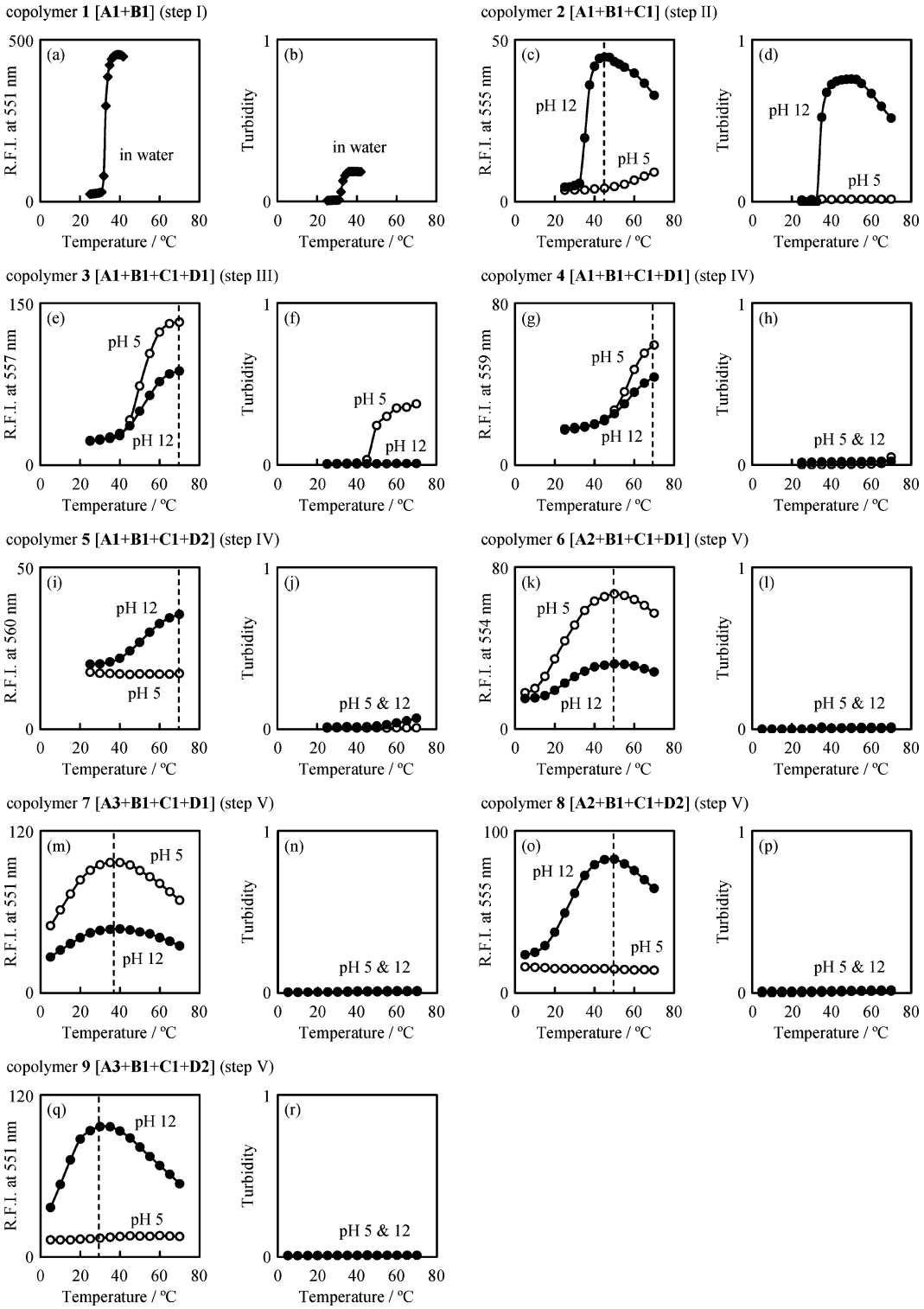

Figure 2. Functionalization of copolymers. Relative fluorescence intensity (R.F.I.) (a, c, e, g, i, k, m, o, and q) and turbidity (b, d, f, h, j, l, n, p, and r) of **1**−**9** (0.01 w/v%) in water at different temperatures. Details of each functionalization step (I−V) are given in the text. Britton−Robinson buffer was used for pH 5 and pH 12. The excitation wavelength was 450 nm.

following dehydration.[9] Thus, the microenvironmental polarity near the main chain of PNIPAM decreases with increasing temperature. On the other hand, comonomer **B1** possesses a benzofurazan fluorophore, which fluoresces strongly in a less polar environment.[8a] Therefore, in the case of **1**, a strong fluorescence signal is observed only above 32 °C, i.e., under conditions affording the globular-shaped structure of the copolymer. The turbidity of **1** also increased with increasing temperature (Figure 2b). This phase behavior of **1** is similar to that of PNIPAM, indicating that the incorporation of a small amount of **B1** unit did not affect the characteristics of PNIPAM.

Incorporation of a H^+-Binding Unit into the Copolymer 1, Producing a Fluorescence Response to H^+ (step II). Next, *N*,*N*-dimethylaminopropylacrylamide (**C1**) as a proton receptor was added to the feed to afford copolymer **2** ([**A1**/**B1**/**C1**] = [90/0.1/10]). Considering that the pK_a value of trimethylamine is 9.8, the fluorescence and turbidity measurements were performed at pH 12, where the **C1** units in the copolymer **2** are

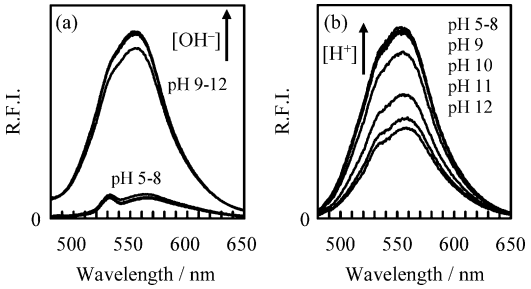

Figure 3. (a) Fluorogenic response of **2** to OH^- at 45 °C in aqueous solution (0.01 w/v%). The peak at 530 nm is due to Raman scattering from water. (b) Fluorogenic response of **6** to H^+ at 50 °C in aqueous solution (0.01 w/v%). The excitation wavelength was 450 nm.

neutral, and at pH 5, where they are fully protonated. At pH 12, the changes in fluorescence intensity and turbidity of **2** were large in the range of 25 to 45 °C (Figure 2c,d).[12] This suggests that **2** changed its conformation from an open form to a globular form with increasing temperature, in a similar manner to the case of the copolymer **1**. On the other hand, at pH 5, only a small change in fluorescence intensity and no change in turbidity were observed, in spite of the increase in temperature. This indicated that **2** remained in an open form, since the protonated **C1** units at pH 5 made the copolymer **2** too hydrophilic to take a globular form even at high temperature.

From the viewpoint of fluorescent sensors, **2** functions as a fluorogenic OH^- sensor at around 45 °C, as shown in Figure 3a, whereas our first goal is to create a fluorogenic H^+ sensor. The fluorescence intensity of copolymer **2** at pH 12 was about 9 times higher than that at pH 5. As shown in Figure 3a, the maximum emission wavelength of the fluorescent **B1** unit in **2** was shifted from 566 to 555 nm with increasing OH^-. This blue shift indicated that there is a decrease in the local polarity around the **B1** unit[8a] due to the structural change of the copolymer from an open form to a globular form in the presence of OH^-, which is consistent with our proposed mechanism.

Incorporation of an Anionic Unit into the Copolymer 2, Reversing the Fluorescence off-on Response to H^+ (Step III). The introduction of the polarity-sensitive fluorescent comonomer **B1** and the H^+-binding comonomer **C1** into a copolymer (i.e., copolymer **2**) resulted in a fluorescence response to H^+, but the fluorescence was quenched with increasing H^+ (i.e., on−off action, see Figure 2c), which was opposite to our desired fluorogenic response (i.e., off-on action). Thus, anionic 2-acrylamido-2-methylpropanesulfonic acid (**D1**) was added to the feed to afford copolymer **3** ([**A1**/**B1**/**C1**/**D1**] = [90/0.1/5/5]). The incorporation of the anionic **D1** is expected to reverse the fluorescence response to H^+. As shown in Figure 2e, fluorescence enhancement of copolymer **3** with increasing temperature was observed at both pH 5 and pH 12. The enhancement at pH 5 was larger than that at pH 12, which was the opposite response to that found in **2**. The turbidity of **3** increased only at pH 5 (Figure 2f), which was also opposite to the response of **2**. At pH 12, the copolymer **3** is negatively charged overall, due to the presence of the electrically neutral **C1** unit under basic conditions and the anionic **D1** units. On the other hand, at pH 5, the net charge of **3** is almost neutral owing to the electrostatic attraction between the anionic **D1** units and the protonated **C1** units (the ratios of **D1** and **C1** in feed were equal, 5%). Therefore, **3** becomes more hydrophobic under acidic conditions, favoring a globular form. In this case, **3** showed 1.5 times stronger fluorescence at pH 5 than that at pH 12, at 70 °C. The incorporation of the anionic **D1** unit is, thus, an important step toward developing the desired fluorogenic H^+ sensor.

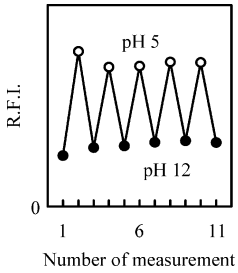

Figure 4. Reversibility of fluorescence signal of **6** between pH 12 (closed circle) and pH 5 (open circle). Sodium hydroxide and hydrochloric acid were used to adjust the pH.

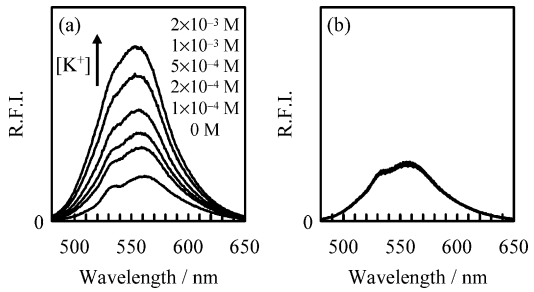

Figure 5. Fluorescence responses of (a) **10** and (b) **11** to K^+ at 40 °C in water (0.01 w/v%). The turbidity of each solution was unchanged during the measurement. Potassium chloride was used as a K^+ source. Notably, potassium hydroxide as a K^+ source gave similar results. The excitation wavelength was 450 nm.

Incorporation of an Additional Ionic Unit, Increasing the Solubility of Fluorogenic Ion Sensors (Step IV). The copolymer **3** is not completely soluble in water, as was indicated by the turbidity increase (see Figure 2f). For applications to biomatrices or environmental samples, high solubility of fluorogenic ion sensors in water is advantageous. To increase the solubility of the sensor in water, an excess amount of ionic **D1** with respect to **C1** was used in the feed for copolymer **4** ([**A1**/**B1**/**C1**/**D1**] = [88/0.1/5/7]). Now, the net charge of **4** is not neutral even under acidic conditions, since the amount of protonated **C1** units (5% in feed) is less than that of the anionic **D1** units (7% in feed). As expected, **4** did not exhibit turbidity, regardless of pH changes (Figure 2h), while the fluorescence response to H^+ was seen at around 70 °C (i.e., the fluorescence intensity at pH 5 is 136% higher than that at pH 12, Figure 2 g). These results mean that the copolymer **4** can be regarded as a fluorogenic H^+ sensor with high solubility in water, though it is not highly sensitive to change of pH. Such a microscopic response of a stimulus-responsive polymer without a turbidity increase has been little explored. The incorporation of a polarity-sensitive fluorophore into a stimulus-responsive polymer enables us to visualize a microscopic response without any turbidity increase.

A similar strategy can be applied to obtain a fluorogenic OH^- sensor based on the copolymer **2**. A cationic monomer, (3-acrylamidopropyl)trimethylammonium (**D2**) chloride, was added to the feed for copolymer **2** to obtain copolymer **5** ([**A1**/**B1**/**C1**/**D2**] = [85/0.1/10/5]). The cationic **D2** units in **5** improved the solubility under basic conditions. The responses of fluorescence intensity and turbidity to pH change (Figure 2i,j) showed that the copolymer **5** is a fluorogenic OH^- sensor functioning with high aqueous solubility.

Replacement of the *N*-Isopropylacrylamide (A1) Unit with Other *N*-Alkylacrylamide Units, Regulating Functional Temperature (Step V). Finally, we examined regulation of the functional temperature of fluorogenic ion sensors. We replaced *N*-isopropylacrylamide (**A1**) with *N*-*tert*-butylacrylamide (**A2**),

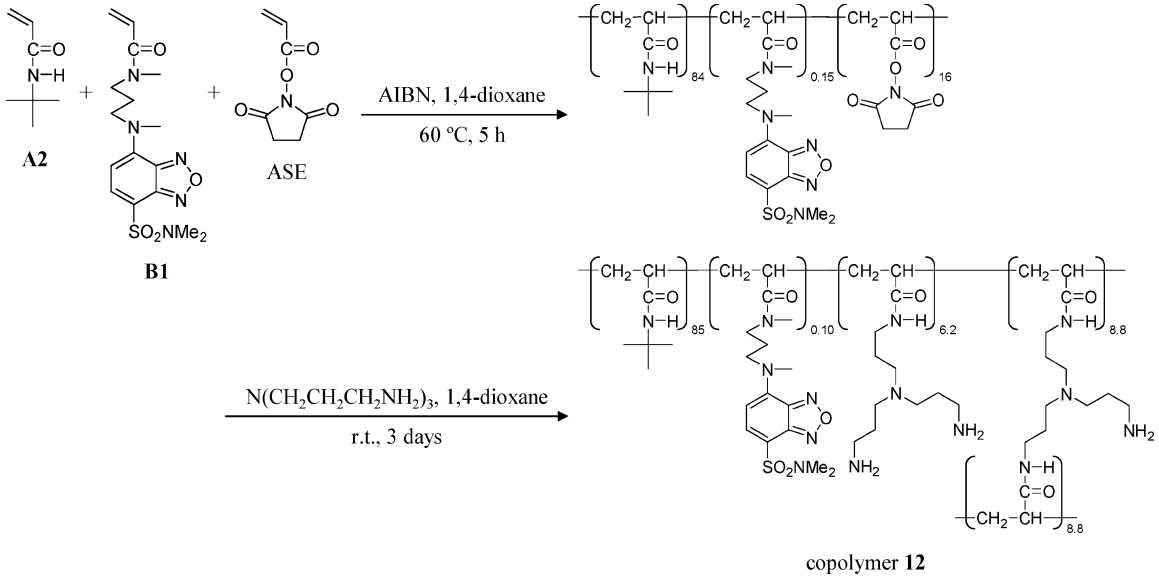

Figure 6. Synthesis of **12**.

bearing a more hydrophobic alkyl group,[13] and obtained copolymer **6** ([**A2**/**B1**/**C1**/**D1**] = [57/0.1/20/23]). As shown in Figure 2k, the functional temperature of **6** as a fluorogenic H^+ sensor was 50 °C, which was lower than that of 70 °C for copolymer **4**. The fluorescence response of **6** to H^+ at 50 °C is shown in Figure 3b. Similarly, when *N*-*tert*-amylacrylamide (**A3**), a more hydrophobic unit than **A2**,[13] was used, the resultant copolymer **7** ([**A3**/**B1**/**C1**/**D1**] = [47/0.1/25/28]) worked as a fluorogenic H^+ sensor with a functional temperature of 37 °C, close to the human physiological temperature (Figure 2m). The order of the functional temperature, **4** (70 °C) > **6** (50 °C) > **7** (37 °C) is consistent with the order of hydrophilicity of the *N*-alkylacrylamide units in the copolymers, **A1** > **A2** > **A3**. This trend is consistent with that seen for general thermoresponsive polymers.[14]

This method for regulating functional temperature can also be applied to the fluorogenic OH^- sensor **5**. The **A1** unit in the sensor **5** was replaced with the **A2** or **A3** unit to afford copolymer **8** ([**A2**/**B1**/**C1**/**D2**] = [65/0.1/30/5]) or copolymer **9** ([**A3**/**B1**/**C1**/**D2**] = [58/0.1/35/7]), respectively. The functional temperatures of **8** and **9** as fluorogenic OH^- sensors were lowered to 50 °C (Figure 2o) and 30 °C (Figure 2q), respectively, as compared with that of **5** (70 °C, Figure 2i).

Reversibility of the Fluorescence Response of Fluorogenic Ion Sensors. Since we had succeeded in developing fluorogenic H^+ ion sensors (copolymers **3**, **4**, **6**, and **7**), the reversibility of the fluorescence response was investigated. Figure 4 shows the result for the fluorogenic H^+ sensor **6** as a representative; it was confirmed that our fluorogenic sensing system has reversibility and longevity.

Development of a Fluorogenic K^+ Sensor.

Next, we developed a new fluorogenic K^+ sensor functioning in water[15] in order to demonstrate the generality of our design concept. For this purpose, copolymer **10** ([**A2**/**B1**/**C2**/**D1**] = [68/0.1/12/20]) incorporating the K^+-binding **C2** unit[10a] was prepared. The **C2** unit possesses a benzo-18-crown-6 moiety, and the binding constant of this crown ether for K^+ in water was reported to be 1.74 (logarithm).[16] Copolymer **11** ([**A2**/**B1**/**C4**/**D1**] = [68/0.1/12/20]) was also prepared as a negative control, which contained the **C4** unit with low affinity for K^+. The fluorescence responses of **10** and **11** to K^+ at 40 °C are shown in Figure 5. The fluorescence intensity of **10** was significantly higher (413%) in the presence of K^+ (2×10^{-3} M) than that in the absence of K^+ (Figure 5a), whereas the fluorescence intensity of **11** was unchanged regardless of the K^+ concentration (Figure 5b). These results clearly indicate that the copolymer **10** is a fluorogenic K^+ sensor functioning in water through interaction of K^+ with the **C2** units. These results suggested that our buildup design concept is applicable to the development of a new fluorogenic K^+ sensor.

Development of a Fluorogenic SO_4^{2-} Sensor.

Finally, we developed a new fluorogenic sulfate (SO_4^{2-}) sensor in order to confirm the applicability of our concept to fluorogenic anion sensing in water, which is an area of interest to many researchers.[17] The determination of SO_4^{2-} is very important for environmental studies, since it provides information on pollution levels, the purity of drinking water, and so on.[18] It is worth noting that there is little information available about fluorogenic sensing of SO_4^{2-} in water.[19] We chose the **C3** unit as an SO_4^{2-}-binding moiety for a fluorogenic SO_4^{2-} sensor, since the protonated form of the related tris(3-aminopropyl)amino structure can bind SO_4^{2-} in water (the binding constant for SO_4^{2-} is 1.6 (logarithm) at pH 6).[20] Then, we prepared copolymer **12** (corresponding monomer ratio in feed [**A2**/**B1**/**C3**] = [90/0.1/10]) as a new fluorogenic SO_4^{2-} sensor, as shown in Figure 6. The **C3** unit was introduced into the polymer, while some of the tris(3-aminopropyl)amino groups were further acylated. In the case of the copolymer **12**, the incorporation of ionic units **D1** or **D2** was not necessary, since the binding of the protonated **C3** units with SO_4^{2-} decreases the net charge of the copolymer, promoting the structural change from an open form (swelling) to a globular form (shrinkage), and since the positive charges (+3) of the triprotonated **C3** units are not neutralized by one-to-one binding with SO_4^{2-}. In this context, this sensor only works under acidic conditions.

The fluorescence response of **12** to SO_4^{2-} at 25 °C is shown in Figure 7. The fluorescence intensity of **12** was significantly higher (420%) in the presence of SO_4^{2-} than that in the absence of SO_4^{2-}. For fluorescent SO_4^{2-} sensing, 0.01 M hydrochloric acid solution was used as the medium to keep the **C3** units fully protonated and to obtain a solution of **12**. Under these conditions, other tested anions (acetate, chloride, nitrate, and phosphate) did not induce such a fluorescence enhancement (the maximum was 155% for oxalate), because they exist in the neutral form or have weak affinity for the protonated **C3** units. In the presence of 0.01 M hydrochloric acid solution (ap-

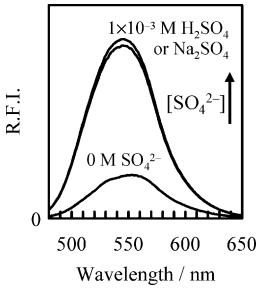

Figure 7. Fluorescence response of **12** (0.01 w/v%) to SO_4^{2-} at 25 °C in 0.01 M hydrochloric acid solution. The turbidity of the solution was unchanged during the measurement. The excitation wavelength was 450 nm.

proximately pH 2), sulfate (SO_4^{2-}) equilibrates with HSO_4^- because the pK_a of HSO_4^- is 2.0. Thus, SO_4^{2-} and HSO_4^- coexisted in the present solution. In the previous report,[20] monoanionic dimethyl phosphate exhibited much weaker binding to the tris(3-aminopropyl)amino structure (in the triprotonated form) compared with phosphate (a mixture of monoanion and dianion). Similarly, it can be reasonably assumed that the triprotonated **C3** unit prefers binding with SO_4^{2-} rather than with HSO_4^-. Thus, our design concept for fluorogenic ion sensors, i.e., the combination of a stimulus-responsive polymer and a polarity-sensitive fluorophore, has enabled us to develop the first fluorogenic SO_4^{2-} sensor working in water.

Conclusion

In this work, we describe fluorogenic ion sensors of a new type, based on the combination of a stimulus-responsive polymer and a polarity-sensitive fluorophore. In aqueous solution, our fluorogenic ion sensors change their three-dimensional structures from an open form to a globular form at the functional temperature upon binding the target ion, resulting in a fluorescence enhancement of the polarity-sensitive fluorophore via the decrease in the local polarity. The function of the sensor can be easily tuned in terms of target ion and functional temperature by selecting suitable combinations of comonomers from some or all of the four types, in appropriate ratios. Moreover, our sensors work without requiring a well-defined alignment of the various type of comonomer units in the overall structure. We believe this represents a new general design approach to fluorogenic ion sensors functioning in water.

Experimental Section

General Data. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a JEOL JNM-GX270 spectrometer or a BRUKER AVANCE 400 spectrometer. The *J* values are given in hertz. Mass spectra were measured using VG Autospec-X spectrometers. High-resolution mass spectra (HRMS) were obtained by a peak-matching method using perfluorokerosene as a standard. UV−vis absorption spectra and transmittance were measured using a JASCO V-550 UV/vis spectrophotometer with a JASCO ETC-505 temperature controller. Fluorescence spectra were measured using a JASCO FP-6500 spectrofluorometer with a JASCO ETC-273 temperature controller. The gel permeation chromatography (GPC) equipment consisted of a JASCO DG-2080−53 degasser, a JASCO PU-2080 pump, a JASCO CO-2060 column oven, a JASCO RI-2031 refractive index detector, and a Shodex GPC KD-806M column. *N*-Isopropylacrylamide (**A1**) and 2,2′-azobis(isobutyronitrile) (AIBN) were purchased from Wako Pure Chemicals and purified by recrystallization from *n*-hexane and methanol, respectively. 4-*N*,*N*-Dimethylaminosulfonyl-7-fluoro-2,1,3-benzoxadiazole (DBD−F) was purchased from TCI. 4-Acrylamidobenzo-18-crown-6 (**C2**) was purchased from Acros Organics. Acrylic acid *N*-hydroxysuccinimide ester (ASE) was purchased from Sigma. All other reagents and solvents were commercial products and were used as supplied.

Monomer Synthesis. *N-tert*-Amylacrylamide (**A3**) was synthesized as previously described.[21]

***N*-{3-[{7-(Dimethylaminosulfonyl)-2,1,3-benzoxadiazol-4-yl}-(methyl)amino]propyl}-*N*-methylacrylamide (B1).** DBD−F (100 mg, 0.41 mmol) was dissolved in acetonitrile (5 mL), and the solution was added to *N*,*N*′-dimethyl-1,3-propanediamine (1.5 mL, 12 mmol). The mixture was stirred at room temperature for 2 h, then evaporated to dryness under reduced pressure, and the residue was chromatographed on silica gel with dichloromethane−methanol (10 : 1 to 5 : 1 to 4 : 1) to afford an orange oil (115 mg, 0.35 mmol). This oil (110 mg, 0.34 mmol) was dissolved in acetonitrile (10 mL). To this solution were added triethylamine (46.8 μL, 0.34 mmol) and acryloyl chloride (35.5 μL, 0.44 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h. Then K_2CO_3 (1 g) was added. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel with dichloromethane-methanol (20 : 1) to afford **B1** (118 mg, 0.31 mmol, 78% in two steps) as an orange oil. ^{1}H NMR ($CDCl_3$): δ 7.86 (1H, d, J = 8.3), 6.63 (1H, m), 6.35 (1H, m), 6.04 (1H, d, J = 8.3), 5.73 (1H, m), 4.07 (2H, m), 3.56 (2H, m), 3.29 (3H, m), 3.14, 3.05 (3H, s), 2.87 (6H, s), 2.02 (2H, m). HR−CI−MS, *m*/*z*: calcd for $C_{16}H_{24}N_5O_4S$ ([M + H]$^+$), 382.1549; found, 382.1533.

***N*-(3,4-Dimethoxyphenyl)acrylamide (C4).** 3,4-Dimethoxyaniline (1.00 g, 6.5 mmol) was dissolved in acetonitrile (15 mL). To this solution, triethylamine (906 μL, 6.5 mmol) and acryloyl chloride (530 μL, 6.5 mmol) were added at 0 °C, and the mixture was stirred at 0 °C for 40 min. To the reaction mixture was added saturated aqueous Na_2CO_3 (100 mL), and the entire product was extracted with dichloromethane (100 mL × 2). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure, and the residue was chromatographed on silica gel with *n*-hexane−ethyl acetate (1:2) to afford **C4** (1.11 g, 5.4 mmol, 82%) as colorless needles. Mp: 106−107 °C (recrystallized from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 7.48 (1H, d, J = 2.0), 6.91 (1H, dd, J = 2.0, 8.4), 6.81 (1H, d, J = 8.4), 6.43 (1H, dd, J = 0.8, 16.8), 6.23 (1H, dd, J = 10.0, 16.8), 5.76 (1H, dd, J = 0.8, 10.0), 3.89 (3H, s), 3.87 (3H, s). Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.71; H, 6.49; N, 6.79.

Polymerizations. All units were introduced by means of radical polymerization and the composition was optimized for effective function.

Copolymers 1—11. B1 (0.50 mM) and the other acrylamide monomers (total 0.50 M, feed composition as shown in Table 1), together with AIBN (5.0 mM), were dissolved in 5−10 mL of 1,4-dioxane (**1** and **2**), DMF (**3**−**5** and **8**−**11**) or methanol (**6** and **7**). The dissolved oxygen was removed by N_2 bubbling for 20−30 min. The solution was heated to 60 °C for at least 12 h and then cooled to room temperature. The reaction mixture was poured into diethyl ether, and the precipitate was purified by reprecipitation and/or dialysis. The yields are listed in Table 1.

Copolymer 12. A2 (0.45 M), **B1** (0.50 mM), ASE (0.050 M), and AIBN (5.0 mM) were dissolved in 1,4-dioxane (10 mL). The dissolved oxygen was removed by N_2 bubbling for 50 min. The solution was heated to 60 °C for 5 h and then cooled to room temperature. The reaction mixture was poured into diethyl ether, and the precipitate was purified by reprecipitation using 1,4-dioxane and diethyl ether to afford copolymer ([**A2**/**B1**/ASE] = [90/0.1/10]) (42%, $M_w = 1.17 \times 10^5$ and $M_n = 7.12 \times 10^4$). The obtained copolymer (150 mg) was dissolved in 1,4-dioxane (4 mL). The solution was gradually added to tris(2-aminopropyl)amine (TAPA, 2 mL) in 1,4-dioxane (2 mL) and the mixture was stirred at room temperature for 3 days. It was then poured into diethyl ether, and the precipitate was purified by reprecipitation using acetone and *n*-hexane to afford copolymer **12** (15%). The ^{1}H NMR spectrum of copolymer **12** indicated that 74% of the ASE units of the copolymer ([**A2**/**B1**/ASE] = [90/0.1/10]) reacted with TAPA at 2 : 1 ratio (i.e., cross-linking), and 26% of the ASE units reacted with TAPA at 1 : 1 ratio to afford the desired **C3** units.

Characterization of the Synthesized Copolymers. The contents of the monomer units except for the **B1** unit in copolymers **1**−**12** were determined from the ^{1}H NMR spectra. The proportions of the **B1** unit were determined from the measured absorbance in methanol, based on that of 4-*N*,*N*-dimethylamino-7-*N*,*N*-dimethylaminosulfonyl-2,1,3-benzoxadiazole (ϵ = 10 600 $M^{-1}cm^{-1}$ at 442 nm[8a]) as a reference compound. The actual compositions of copolymers **1**−**12** are listed in Table 1. The molecular weights of the copolymers were determined by GPC with 1-methyl-2-pyrrolidinone containing LiBr (5 mM) as an eluent. The weight-average molecular weight (M_w) and number-average molecular weight (M_n) values of copolymers **1**−**12** are also listed in Table 1.

Fluorescence and Turbidity Measurements. The concentration of the copolymers was 0.01 w/v% in aqueous solution during all measurements. The fluorescence spectra were measured with excitation at 450 nm. Turbidity was obtained by using the following equation: turbidity = 1 − [transmittance at 600 nm]. The temperature of the sample solution was monitored with a thermocouple.

Acknowledgment. This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan. M.O. thanks the Japan Society for the Promotion of Science for a JSPS Research Fellowship for Young Scientists. S.U. thanks Kurita Water and Environment Foundation for financial support.

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(12) It should also be noted that the fluorescence intensity of copolymer **2** at pH 12 gradually decreased with increasing temperature over 45 °C. This is not due to the instability of copolymer **2** under these conditions (data not shown, cf. Figure 4, in which the reversibility and longevity of copolymer **6** were confirmed). Since the maximum emission wavelength of the **B1** unit in **2** was essentially constant (555 nm, data not shown) over 45 °C, the decrease in the fluorescence intensity is not due to the heat-induced reverse structural change of the copolymer **2** from a globular form to an open form. Rather, intermolecular quenching (e.g., by electron transfer between the **B1** unit and the other units) or intramolecular quenching (e.g., by intersystem crossing within the **B1** unit) seems to be accelerated at higher temperature.

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MA071641I