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Montmorillonite−Supramolecular Hydrogel Hybrid for Fluorocolorimetric Sensing of Polyamines

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Supporting Information

ABSTRACT: Fluorescent sensor materials for rapidly and conveniently detecting polyamines in biological fluids are highly desirable for cancer diagnosis. We herein describe the hybridization of a supramolecular hydrogel with a layered inorganic host adsorbing a fluorescent dye which produces a fluorocolorimetric sensor for spermine and spermidine, important biomarkers for cancers, in artificial urine.

Polyamines, such as spermine and spermidine, are known to play an important role in cell growth and proliferation and thus are expected to be good biomarkers for abnormally and rapidly growing cancers.[1] For example, it is reported that determination of urinary polyamine concentrations can be used for assessing the effectiveness of cancer chemotherapy.[2] Although these polyamines are currently detected using immunoassays[1b] and chromatographic techniques,[1c,2a] these are time-consuming tedious procedures involving expensive equipments. Convenient diagnostic tools capable of sensing such biomarkers in a rapid, label-free, and high-throughput manner are highly desirable.[3,4]

We recently developed semiwet fluorescent sensor materials by hybridization of a supramolecular hydrogel with an inorganic host encapsulating a fluorescent dye as a probe.[5c] In this gel-based sensor, an anionic fluorescent dye is encapsulated into a cationic mesoporous silica for detecting poly*anions*. We demonstrated that the supramolecular hydrogel not only serves as an immobilization matrix, but also plays an active role in converting signals of the fluorescent dye. Since the self-assembled fibers in the supramolecular hydrogel provide continuous hydrophobic nano-spaces, the fluorescent dye bearing a hydrophobic part can be entrapped inside the fibers upon being released from the host through a selective exchange with the target (guest) substances, which causes fluorescence spectral changes. Thus, the fluorescent dye acts as a probe for following the exchange phenomena.

We herein successfully expanded our strategy toward sensing poly*cations* using a naturally abundant anionic layered material, montmorillonite[6] (MMT), as the host and a cationic fluorescent dye in supramolecular hydrogel **1**[7] (Figure 1). In the present system, it was crucial that the surface of the MMT nanosheet had high anionic charge density to facilitate aggregation of the cationic fluorescent probe.[8] The adsorbed cationic probe showed a weak greenish, excimer emission, which converted to an intensified

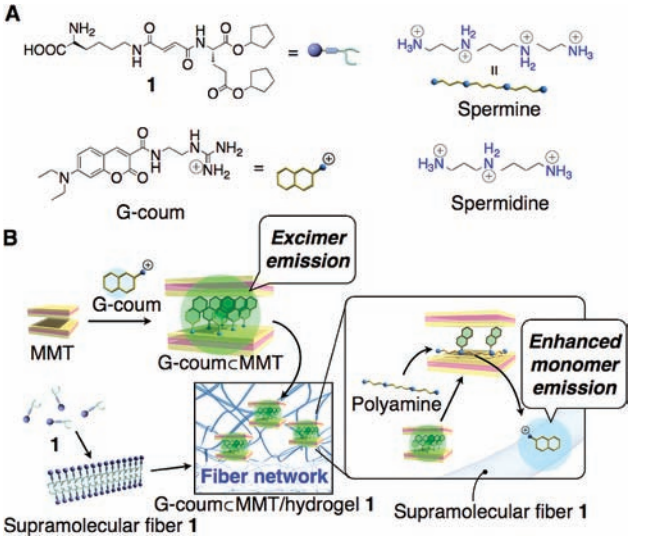

Figure 1. (A) Chemical structures of hydrogelator **1**, G-coum, and polyamines; (B) construction and the mechanism of action of the fluorescence dye (G-coum) adsorbed MMT/supramolecular hydrogel **1** hybrid sensory system for polyamines.

blue, monomer emission through its release via the cation-exchange and the subsequent translocation to the supramolecular fibers **1**. This gave rise to a unique fluorocolorimetric readout mechanism, different from the previous systems.[5] We also demonstrated that an array using the miniaturized hybrid sensor is promising for user-friendly naked eye detection of spermine and spermidine in artificial urine.

The catch and release function of MMT for cationic substances was first investigated as an aqueous suspension system. Adsorption of a cationic coumarin dye (G-coum) bearing a guanidium group to MMT occurred almost quantitatively by mixing G-coum and MMT in neutral aqueous media (Figure S1A). Importantly, the adsorbed G-coum to MMT (G-coum⊂MMT) was released by the addition of spermine (28 ± 1%) but not by propylamine (1.3 ± 0.4%).[9] As summarized in Figure 2A, it was clear that the release was efficiently induced by polyamines such as spermine and spermidine and several diamines,[10] but not by propylamine (monoamine), urea, or ATP (adenosine triphosphate),

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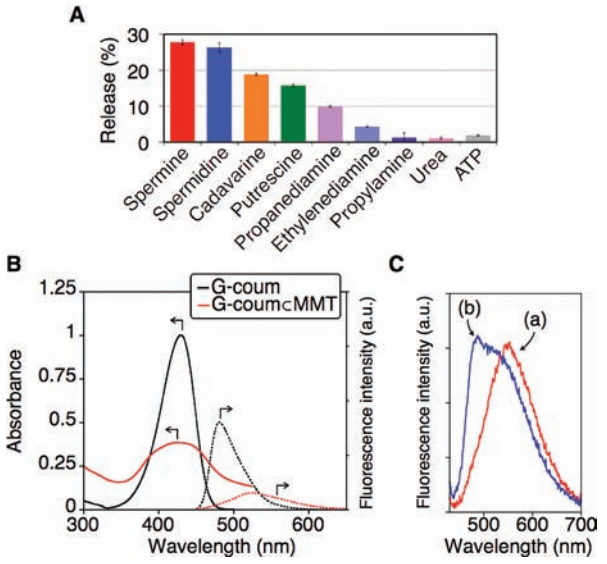

Figure 2. (A) Release of G-coum from MMT upon the addition of various substances. Conditions: [G-coum⊂MMT] = 1.0 mg/mL, [G-coum] = 0.23 μmol/mg of MMT (19% CEC), [Substances] = 10 mM, 50 mM HEPES (pH 7.4). (B) Absorption and fluorescence (λ_{ex} = 360 nm) spectra of aqueous G-coum solution ([G-coum] = 117 μM) and aqueous G-coum⊂MMT suspension ([G-coum⊂MMT] = 0.50 mg/mL, [G-coum] = 0.23 μmol/mg of MMT (19% CEC), 50 mM HEPES (pH 7.4)) at room temperature. (C) Fluorescence spectral change (λ_{ex} = 410 nm, MCPD (multichannel photodetector), see Supporting Information for details) of G-coum⊂MMT suspension (a) before and (b) after the addition of spermine. Conditions: [G-coum⊂MMT] = 0.40 mg/mL, [G-coum] = 0.23 μmol/mg of MMT (19% CEC), [Spermine] = 0.91 mM, 50 mM HEPES (pH 7.4).

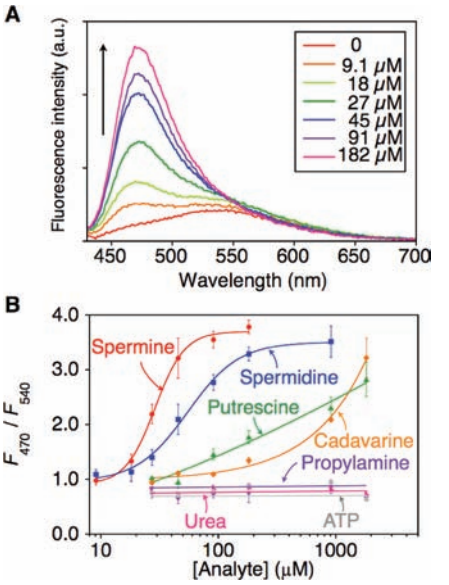

Figure 3. (A) Fluorescence spectral change (λ_{ex} = 410 nm, MCPD) of G-coum⊂MMT/hydrogel **1** upon the addition of spermine and (B) fluorescence titration curves (see also Figure S3 for the spectral changes). Lines for spermine and spermidine are best fit of Hill equation to data points. Error bars represent standard deviation (n = 3). Conditions: [**1**] = 0.18 wt %, [G-coum⊂MMT] = 0.40 mg/mL, [G-coum] = 0.23 μmol/mg of MMT (19% CEC), 50 mM HEPES (pH 7.4).

indicating that substances having more than two positive charges can facilitate the cation-exchange reaction of G-coum with MMT.

Noteworthy was the fact that the fluorescence spectra of the G-coum adsorbed to MMT[11] (G-coum⊂MMT) displayed a significantly quenched, red-shifted emission[12] (λ_{max}: from 470 to 540 nm) compared with those of G-coum in an aqueous solution (Figure 2B). Concurrently, a broadened absorption band (fwhm: from 51 to 88.5 nm) was observed for G-coum in MMT. As shown in Figure 2C (from (a) to (b)), it is interesting that the excimer emission at 540 nm decreased, whereas the monomer emission at 470 nm increased by the addition of spermine. These results suggest that G-coum was adsorbed and aggregated on the anionic surface of MMT at high loading levels compared to its CEC (cation-exchange capacity) and the aggregated G-coum in MMT was released into the aqueous phase as a monomeric form through the cation-exchange with spermine.

More importantly, the blue-shifted fluorescence of G-coum released by spermine was significantly intensified when we embedded the G-coum⊂MMT in a supramolecular hydrogel matrix (G-coum⊂MMT/hydrogel **1** hybrid) (Figure 3A). The fluorescence intensity at 470 nm by the addition of spermine was 5.0-fold greater than that in aqueous medium without hydrogel **1** (Figure 2C). This type of fluorescence spectral change, from the excimer to the enhanced monomer emission, can be ascribed to both the environmentally sensitive nature of the G-coum fluorescence[13] and the binding capacity of the supramolecular fiber. That is, the G-coum released from MMT by the exchange with spermine was spontaneously translocated from the aqueous phase to the hydrophobic space of supramolecular fibers **1**, where G-coum showed the intensified monomeric emission.[13] Confocal laser scanning microscopy supported the spermine-induced translocation of G-coum as follows. In hydrogel **1**, the fluorescence of G-coum⊂MMT was observed as fluorescent spots of 1−10 μm in diameter (Figure S5A), indicating that G-coum was predominantly bound to MMT even in the hydrogel **1**. It was also evident that MMT was segregated from the supramolecular fibers **1** stained by a hydrophobic BODIPY dye. Clearly, these two microdomains are orthogonal to each other in the semiwet matrix. After the addition of spermine, the segregated spots due to G-coum became smeared and more importantly the G-coum fluorescence was localized along the fibers **1** and overlapped significantly with the BODIPY fluorescence (the merged image, Figure S5B).

The large change in fluorescence intensity along with the emission peak shift of the G-coum⊂MMT/hydrogel **1** hybrid upon the addition of spermine enabled us to accurately monitor the spermine concentration by two-wavelength emission ratiometry. As shown in Figure 3B, plotting the fluorescence intensity ratio between the monomer and the excimer (F_{470}/F_{540}) as a function of spermine concentration showed a typical saturation behavior, which clearly validates the fluorescence sensing ability of G-coum⊂MMT/hydrogel **1** hybrid for spermine. From the fluorescence titration experiments of various substances (Figure 3B), it was demonstrated that spermine and spermidine can be detected in the range of 20−100 μM (EC_{50} (the analyte concentration inducing 50% of the maximum signal change) = 29 ± 1.4 and 55 ± 4.8 μM for spermine and spermidine, respectively), whereas propylamine, urea, and ATP were not detected because of their low cation-exchange capability. This order is in good agreement with that obtained in the aqueous G-coum⊂MMT suspension system, indicating the cation selectivity of MMT remained even after the hybridization of supramolecular hydrogel.

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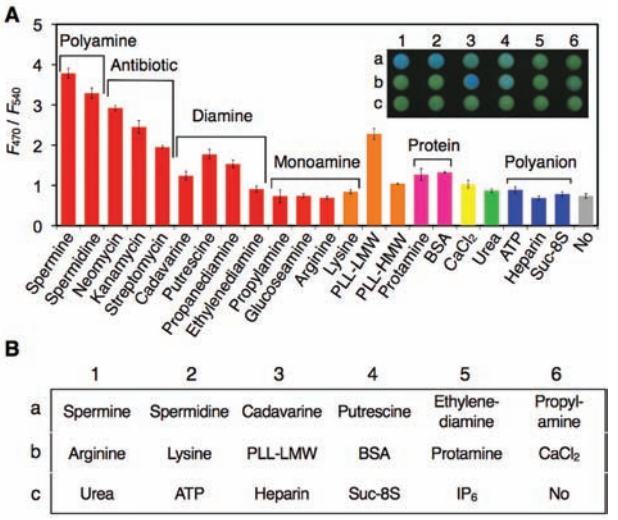

Figure 4. (A) Change in fluorescence intensity ratio (F_{470}/F_{540}) upon the addition of various substances. Inset shows photograph of G-coum⊂MMT/hydrogel **1** hybrid sensor chip (λ_{ex} = 365 nm) for high-throughput sensing of a variety of substances and the spotted position of the substances is shown in panel B. Conditions: spotted volume = 22 μL, [**1**] = 0.18 wt %, [G-coum⊂MMT] = 0.40 mg/mL, [G-coum] = 0.23 μmol/mg of MMT (19% CEC), [Substance] = 182 μM, 50 mM HEPES (pH 7.4).

Figure 4A summarizes the sensing selectivity of G-coum⊂MMT/hydrogel **1** for various substances on the basis of the changes in the fluorescence intensity ratio (F_{470}/F_{540}). It is clear that polyamines such as spermine and spermidine were detected the best, while aminoglycoside antibiotics such as neomycin and kanamycin and poly(L-lysine)[14] (low M_w: PLL-LMW) were also detectable effectively, but diamines with spacers longer than C_2 and proteins such as BSA (bovine serum albumin) were detected with only moderate sensitivity. By contrast, monoamines, ethylenediamine, anions such as ATP, heparin, and sucrose-octasulfate (Suc-8S), and neutral substances such as urea were not detectable. There was little difference in the sensing selectivity between pH 6.8 and 7.4. However, the sensing selectivity was lowered at pH 8.0 most probably due to partial deprotonation of polyamines (Figure S6). Interestingly, these selective and large fluorescence peak shifts enabled us to distinguish polyamines with the naked eye in a high-throughput manner using a G-coum⊂MMT/hydrogel **1** hybrid sensor array chip. As shown in an inset of Figure 4A, intensified blue fluorescence spots for spermine (a1), spermidine (a2), putrescine (a4), and PLL-LMW (b3) were observed, whereas other spots were weaker green for polyanions such as heparin (c3), Suc-8S (c4), and inositol hexaphosphate (IP_6, c5).

To demonstrate the potential in the practical utility of this hybrid sensor material, we finally conducted fluorocolorimetric imaging of spermine and spermidine in artificial urine by using the hybrid sensor array chip. It is proposed that changes in urinary spermine and spermidine concentrations are critical in the range from 1 to 10 μM and 10 to 50 μM, respectively, for cancer diagnosis.[1,15] As shown in Figure 5A, changes in the fluorescence colors were clearly distinguished from green to blue for spermidine (lane a) indeed in such a concentration range (0−45 μM) and for spermine (lane b) in a slightly higher concentration range (0−27 μM), relative to such criteria. The color change required only a few minutes, and both the pixel intensity ratios (I_{blue}/I_{green}) of the digital photograph and the fluorescence

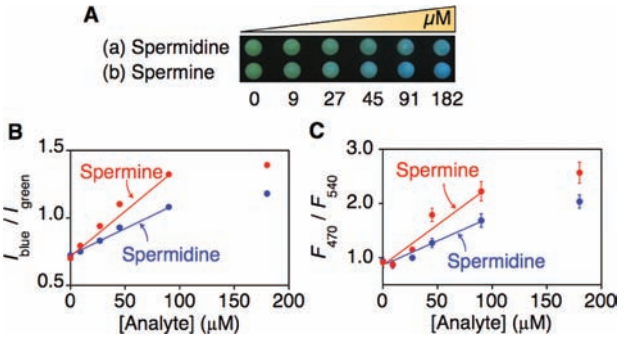

Figure 5. Photograph of G-coum⊂MMT/hydrogel **1** hybrid sensor chip (λ_{ex} = 365 nm) for (A) fluorocolorimetric sensing of (a) spermidine and (b) spermine in artificial urine and the corresponding changes in (B) pixel intensity ratio (I_{blue}/I_{green}) (see Figure S7 for the details) and (C) fluorescence intensity ratio (F_{470}/F_{540}, MCPD). Conditions: spotted volume = 22 μL, [**1**] = 0.18 wt %, [G-coum⊂MMT] = 0.40 mg/mL, [G-coum] = 0.23 μmol/mg of MMT (19% CEC), 50 mM HEPES (pH 7.4).

intensity ratios (F_{470}/F_{540}) of the fluorescence spectra almost linearly increased (Figure 5B,C). These results suggest that the present hybrid fluorescent sensor array is tolerant of biological fluids and substances such as serum albumin (HSA) and creatinine (Figures S8, S9) and could be rapid and sensitive enough for diagnosis applications.

We succeeded in developing a new hybrid material of G-coum⊂MMT/supramolecular hydrogel **1** that can fluorocolorimetrically sense polyamines in a biological fluid mimic, achieving rapid and naked-eye detection. The sensing sensitivity was almost in the range demanded for cancer diagnosis and clinical usage, although further practical tests for verification are required. The present results also demonstrated that the rational hybridization of inorganic hosts with supramolecular hydrogel is a general approach for designing sensor systems for a variety of analytes. Construction of not only sensors but also controlled drug release materials may be attractive using this type of hybrid soft materials. Further research on this topic is currently in progress.

■ ASSOCIATED CONTENT

Supporting Information. Figures S1−S9, synthetic procedure of G-coum, and chemical structures of BODIPY dye and analyzed substances. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Release efficiency did not show strong dependency on %loading (in a range of 4−19% loading against CEC of MMT (1.19 μmol/mg)) (Figure S1B).

(10) Ethylenediamine would not act as dication but mainly as monocation due to its low second pK_a (pK_{a1} = 9.93, pK_{a2} = 6.85). Ozaki, H.; Nakamura, A.; Arai, M.; Endo, M.; Sawai, H. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1981–1987.

(11) G-coum was adsorbed to MMT at 19% loading against CEC. At lower loadings such as 4%, both the monomer (470 nm) and the excimer emission (540 nm) were observed (Figure S2). Loading was limited to ca. 20% of CEC to avoid flocculation at higher loadings.

(12) Excitation spectra of the excimer emission peak showed a peak at 395 nm with a shoulder at 444 nm (Figure S2C), which is shifted compared with the absorption band of the monomeric form (430 nm), indicating that G-coum combined with MMT interacts with each other in the ground state.

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(14) The monomer unit concentration was set to be the same for poly(L-lysine)s. Increase in the total concentration of poly(L-lysine), (high M_w: PLL-HMW) induced a larger change in the fluorescence intensity ratio (data not shown).

(15) A standard urinary creatinine concentration of 1.3 mg/mL was used for the calculations. Urinary creatinine concentration is widely used as a dilution marker of the urine sample.