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Construction of Chemical-Responsive Supramolecular Hydrogels from Guest-Modified Cyclodextrins

Wei Deng, Hiroyasu Yamaguchi, Yoshinori Takashima, and Akira Harada*[a]

Abstract: A methodology for preparing supramolecular hydrogels from guest-modified cyclodextrins (CDs) based on the host–guest and hydrogen-bonding interactions of CDs is presented. Four types of modified CDs were synthesized to understand better the gelation mechanism. The 2D ROESY NMR spectrum of β-CD-AmTNB (Am = amino, TNB = trinitrobenzene) reveals that the TNB group was included in the β-CD cavity. Pulsed field gradient NMR (PFG NMR) spectroscopy and AFM show that β-CD-AmTNB formed a supramolecular polymer in aqueous solution through head-to-tail stacking. Although β-CD-AmTNB did not produce a hydrogel due to insufficient growth of supramolecular polymers, β-CD-CiAmTNB (Ci = cinnamoyl) formed supramolecular fibrils through host–guest interactions. Hydrogen bonds between the cross-linked fibrils resulted in the hydrogel, which displayed excellent chemical-responsive properties. Gel-to-sol transitions occurred by adding 1-adamantane carboxylic acid (AdCA) or urea. ^{1}H NMR and induced circular dichroism (ICD) spectra reveal that AdCA released the guest parts from the CD cavity and that urea acts as a denaturing agent to break the hydrogen bonds between CDs. The hydrogel was also destroyed by adding β-CD, which acts as the competitive host to reduce the fibrils. Furthermore, the gel changed to a sol by adding methyl orange (MO) as a guest compound, but the gel reappeared upon addition of α-CD, which is a stronger host for MO.

Keywords: cyclodextrins · host–guest systems · gels · self-assembly · supramolecular chemistry

Introduction

The design and preparation of molecular hydrogels have attracted increasing attention due to their intrinsic scientific interest and technological applications. Elegant hydrogels have been reported to lead to a new polymeric system with remarkable properties.[1] In particular, supramolecular gels derived from small organic molecules, which are held together by noncovalent bonds and are sensitive to external stimuli such as heat, light, pH, and chemicals, have potential as biodegradable materials in drug-delivery systems and chemical sensors.[2] Therefore, the design and preparation of novel hydrogels, especially stimuli-responsive hydrogels, are challenges in the fields of both materials science and medicine.

The gelation of small organic molecules usually progresses as follows: 1) The subunits initially self-assemble to give long, polymer-like fibrils. 2) Then, the elongated fibrils entangle to form a three-dimensional intertwined network, which immobilizes solvent molecules on a macroscopic scale. The fibrils and networks involve a combination of noncovalent interactions such as host–guest interactions and hydrogen bonding. It is well-known that cyclodextrin (CD) incorporates various guest compounds into its cavity by hydrophobic interactions to form inclusion complexes in aqueous media.[3] We previously reported numerous types of supramolecular polymers derived from modified CDs.[4] Additionally, the CD units can gather together by hydrogen bonds due to the abundant OH groups in the CD ring. Numerous groups have reported that mixtures of CDs with polymers promote supramolecular gel formation.[5] For biological applications and environmental protection, biodegradable gels without a polymeric backbone seem to be advantageous and very useful.[6] Herein, we report novel supramolecular hydrogel formation based on host–guest interactions and hydrogen bonds. Supramolecular fibrils form

[a] Dr. W. Deng, Dr. H. Yamaguchi, Dr. Y. Takashima, Prof. A. Harada
Department of Macromolecular Science
Graduate School of Science, Osaka University
Machikaneyama, Toyonaka
Osaka, 560-0043 (Japan)
Fax: (+81) 6-6850-5446
E-mail: harada@chem.sci.osaka-u.ac.jp

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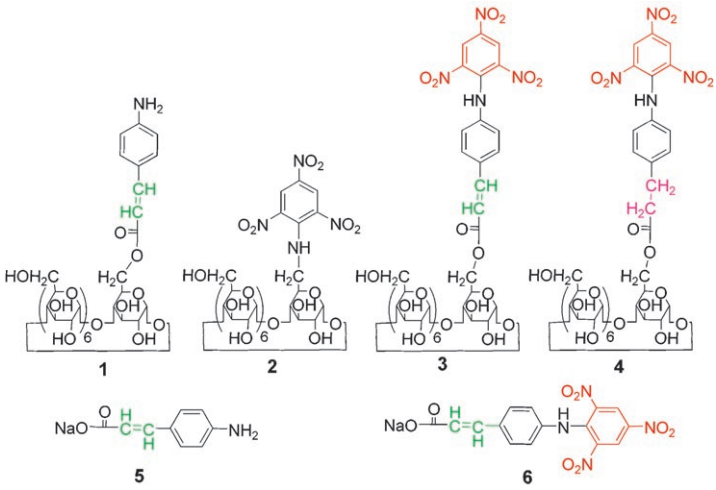
through host–guest interaction, and hydrogen bonds form between the cross-linked fibrils to lead to hydrogels. Therefore, the hydrogels exhibit excellent chemical responsive properties toward competitive guests, hosts, and denaturants.

Results and Discussion

We previously reported that compound **3** forms a supramolecular hydrogel based on host–guest interactions.[7] This discovery led us to refine the gelation method and to develop potential applications. Therefore, several modified CDs were synthesized to understand supramolecular gelation better (Scheme 1). Aminocinnamoyl and 2,4,6-trinitrobenzene (TNB) groups were covalently attached to the 6-position of β-CD to form compounds **1** and **2**, respectively. Compound **4** was also synthesized as a reference compound with flexible hydrocinnamic bridges between CD and the TNB group.



Scheme 1. Chemical structures of compounds **1**–**6**.

These novel modified CDs were dissolved in water at 70 °C to give supersaturated solutions, which were subsequently cooled to room temperature (Figure 1). Compound **1** was insoluble in water because **1** forms intermolecular complexes in a tail-to-tail manner, and the resultant dimers stack tightly through intermolecular hydrogen bonds in a head-to-head channel-type structure.[8] When a supersaturated solution of **2** was cooled to room temperature, a precipitate was obtained as well as compound **4**. However, when the concentration of compound **3** changed from 5 to 10 mM, the clear solution became a mixture of gel and solution. When the concentration of **3** was increased to 20 mM, a red hydrogel was formed with a phase-transition temperature near 50 °C, and the critical gel concentration was calculated to be 2.9 wt %.

To investigate further the supramolecular hydrogel formation based on modified CDs, we then focused on the gelation mechanism. When a guest group is covalently attached to a CD, two types of complexes could form in an aqueous solution through host–guest interaction: an intermolecular complex, which gives a supramolecular dimer or polymer, and an intramolecular complex. With regard to the intermolecular system from a modified CD with a guest, there are two types of stacking, head-to-head (or tail-to-tail) and head-to-tail, which contribute to the formation of a supramolecular dimer and polymer, respectively. On the basis of previous research, compounds **1** and **4** forms the dimer[8] and intramolecular complexes,[9] respectively, both of which inhibit supramolecular polymer formation. Hence, only compounds **2** and **3** were investigated in detail.

Supramolecular Polymer Formation of 2

The ROESY 2D ^{1}H NMR spectrum of **2** shows strong correlations between the protons of TNB and the inner protons of CD in D_2O (Figure 2). Upon adding 1-adamantane carboxylic acid (AdCA), the peak shifted to low field, and the correlations of the inner protons of CD and TNB were replaced by correlations of the inner protons of CD with AdCA protons. These results imply that TNB acts as a guest for β-CD. Figure 3 shows the ^{1}H NMR spectra of **2** at various concentrations in D_2O. As the concentration increased from 4 to 60 mM, the peak of TNB shifted from 9.23 to 9.15 ppm. It was reported that the ^{1}H NMR signal showed high-field shifts when the TNB group was included in another β-CD cavity in water.[3b] Observations of remarkable

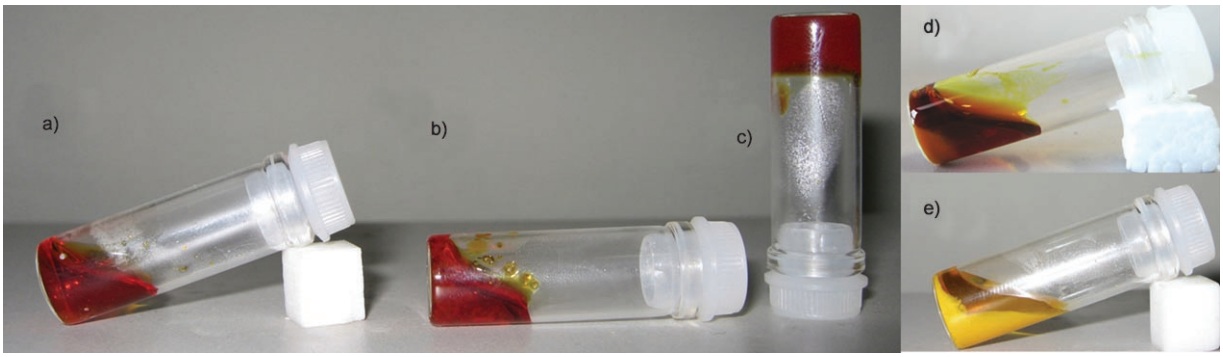


Figure 1. Photograph of **3** at a) 5, b) 10, and c) 20 mM, and precipitates of d) **2** and e) **4** from a supersaturated solution in water at 25 °C.

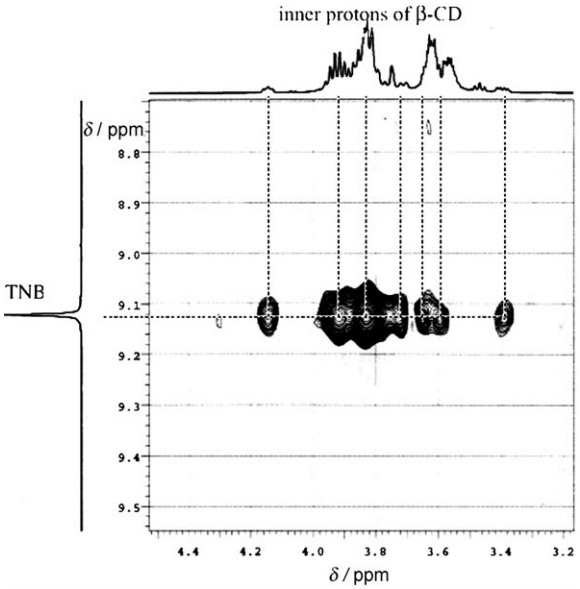


Figure 2. 2D ROESY NMR spectrum (500 MHz) of **2** in D_2O at 30 °C.

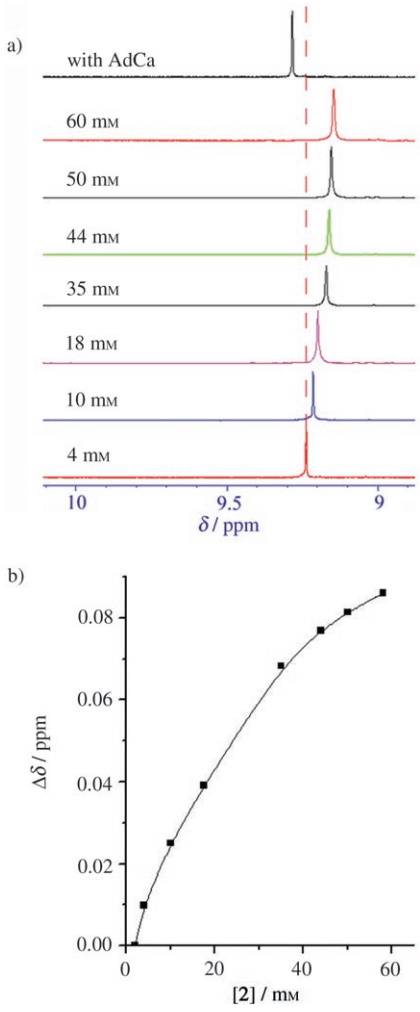


Figure 3. a) ^{1}H NMR spectra of **2** at various concentrations. b) Plot of the change in chemical shift of the TNB peak against concentration of **2** in D_2O at 30 °C.

peak shifts indicate intermolecular complex formation of **2** in aqueous solution.

The self-diffusion coefficients of **2** can be determined by utilizing pulsed field gradient (PFG) NMR spectroscopy in D_2O.[10] According to Stejskal and Tanner,[12] when a graph of ln (I/I_0) versus g^2 is plotted, in which I and g are the echo intensity and (pulsed) gradient strength, respectively, the slope of the line is given by $D/[(\Delta-\delta/3)\gamma^2\delta^2]$. Here, D, δ, γ, and Δ are the diffusion coefficient, the duration, the magnetogyric ratio, and the time interval between the magnetic field gradient pulses, respectively. The diffusion coefficient of native β-CD was reported to be $(3.18\pm0.1)\times10^{-6}$ cm^{2}s^{-1}. Figure 4 is a plot of the diffusion coefficients derived from

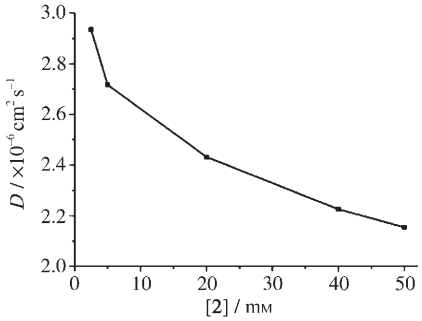


Figure 4. Plot of diffusion coefficient (D) of **2** obtained by PFG NMR spectroscopy as a function of concentration of **2** at 30 °C.

the attenuation of the proton signals of **2** in D_2O as a function of concentration. The diffusion coefficient of **2** decreased from 2.95 to 2.15×10^{-6} cm^{2}s^{-1} when the concentration changed from 2.5 to 50 mM. This result shows that the size of the intermolecular complex formed by **2** increases as its concentration in D_2O increases.

Intermolecular complex formation was directly observed by AFM. Figure 5 shows one of the AFM images of compound **2**; the sample was prepared from an aqueous solution $(5.0\times10^{-8}$ M) on a mica substrate, followed by slow evaporation overnight at room temperature. Several linear objects of compound **2** of different lengths were observed. The

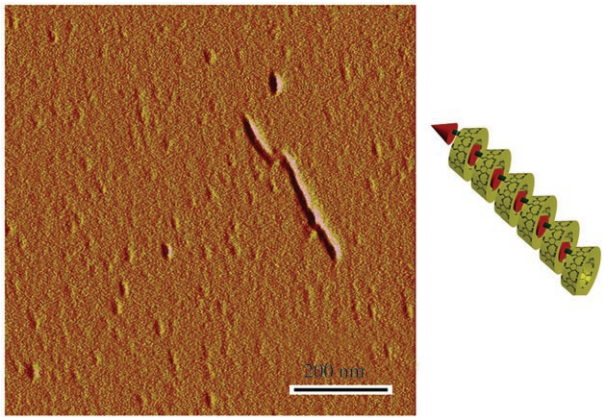


Figure 5. AFM image of **2** from a concentrated aqueous solution $(5.0\times10^{-8}$ M) on mica substrate.

height of the object corresponds to that of the native β-CD ring. These results imply that supramolecular polymer formation occurs from the CD derivative with the 6-position modified by a TNB group. Therefore, modified β-CDs with TNB groups favorably form in the tail-to-head manner for β-CD in aqueous solution due to the space adaptation of TNB with the β-CD cavity.

Supramolecular Polymer Formation of 3

The above experiments confirm that the TNB part acts as a guest for β-CD to form a supramolecular polymer in a tail-to-head manner. Therefore, we modified dimer stack compound **2** with TNB groups to yield compound **3**. Initially, 6-aminocinnamoyl acid salt (**5**) and *N*-(2,4,6-trinitrophenyl)-6-aminocinnamoyl acid salt (**6**) were synthesized as model compounds.

The stoichiometry of the complexation of β-CD with **5** or **6** in aqueous solution was determined by using a Job plot based on the ^{1}H NMR spectral changes. Figure 6 shows the continuous-variation plots of the ^{1}H NMR chemical-shift change for compound **6** ($\Delta\delta$) versus X_{CD}, in which $X_{CD}=[\mathbf{6}]/([\mathbf{6}]+[\beta\text{-CD}])$ and has a maximum value at $X_{CD}=0.5$, which indicates the formation of a 1:1 complex. Similarly, compound **5** also formed a 1:1 inclusion complex with β-CD (see Supporting Information). Additionally, the binding constants K_s were determined by Benesi–Hildebrand plots. The K_s values of β-CD with **5** and **6** were found to be 188 and 850 M^{-1}, respectively. The remarkably enhanced K_s value of **6** is due to the combination of TNB and the cinnamoyl groups.

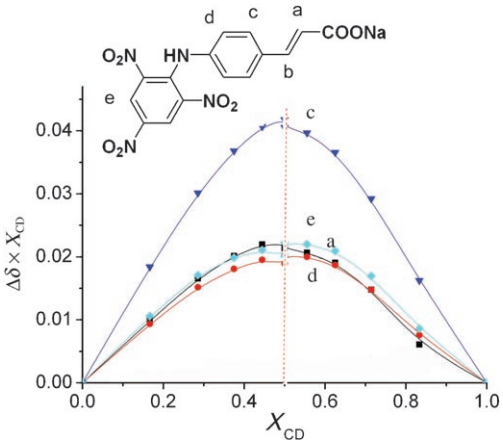


Figure 6. Job plots for the complexation of **6** with β-CD.

Next, structural information about the intermolecular complex formation of **3** was investigated in detail by 2D NMR spectroscopy (see Supporting Information). The 2D ROESY NMR spectrum of **3** shows the ROE enhancements between the inner protons of CD and both the protons of TNB and the cinnamic parts, thus indicating that the guest parts are included in the CD cavities. The H_a and H_b protons of the cinnamoyl moiety showed strong correlations with the protons 3-H and 5-H of β-CD, respectively. Furthermore, the protons of the TNB parts showed correlations with 5-H and 6-H of β-CD, which is indicative of a tail-to-head structure instead of a tail-to-tail structure.

The morphology of **3** was investigated by an optical micrograph and AFM. The observed optical micrograph is characteristic of supramolecular fibrils (see Supporting Information). Figure 7 shows the AFM images of **3** produced from a 5.0×10^{-8} M aqueous solution on mica, followed by drying in air overnight. The large-scale image displays an extraordinarily long linear object, longer than 1 μm. The height of the fibril in the enlarged image is similar to that of native β-CD, which is indicative of a single chain of modified β-CDs. The length of **3** was much longer than that of **2**, and the monomer was not observed, which is consistent with the difference in the affinity of the guests in compounds **2** and **3**. Therefore, modification of the TNB group to CD by a cinnamoyl group not only allows for tail-to-head structure formation, but also greatly enhances the affinity between the guest parts and CD.

Supramolecular Hydrogel Formation

Both **2** and **3** formed supramolecular polymers in water in the above studies, whereas cooling a saturated solution of **2** and **3** gave the precipitate and hydrogel, respectively. The

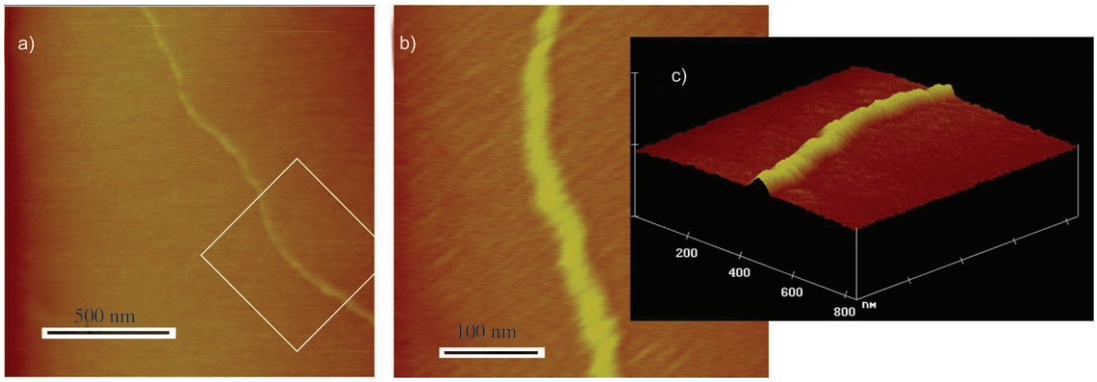


Figure 7. AFM images of **3** from a concentrated aqueous solution (5.0×10^{-8} M) on mica substrate: a) Large-scale image, b) magnified image of the region selected in a), and c) 3D image of b).

difference in the two modified CDs, which give longer and shorter polymer chains, respectively, is that the affinity of the guest to β-CD for **3** is much greater than that for **2**. Figure 8 shows the effect of the concentration of the modified CD on the molecular weight of the supramolecules in water. The molecular weights of **2** and **3** increased with concentration, which implies that **2** and **3** form supramolecular polymers at high concentrations. Vapor pressure osmometry (VPO) measurements of **3** provided evidence that the molecular weight is about 16000 at 5 mM in aqueous solution, which is about 13 times larger than that of the monomer. The molecular weight of **3** is much larger than that of **2** even when the concentration of **2** was as high as 50 mM. These results imply that compound **3** forms much longer supramolecular fibrils. The molecular weights of **4** were about 1300, which is consistent with that of a monomer and is indicative of the formation of the self-inclusion complex in aqueous solution owing to the flexible hydrocinnamoyl group.[12] Therefore, it is concluded that compounds **1**, **2**, and **4** form the dimer crystal, oligomer, and self-inclusion complex in water, respectively, whereas compound **3** forms long polymers that lead to hydrogel formation (Figure 9). These results indicate that formation of a supramolecular polymer with a high molecular weight is important for hydrogel formation. As a comparison experiment, we modified compound **1** with 2,4-dinitrobenzene (DNB) and adamantane-1-carbonyl groups instead of TNB. The DNB group, which is similar to TNB, gave comparable results: a yellow hydrogel was obtained when a saturated solution was cooled, but the adamantane-1-carbonyl-modified compound formed an insoluble dimer, which was the same as **1**. Hence, TNB and the rigid spacer in the guest part play key roles in supramolecular fibril formation.

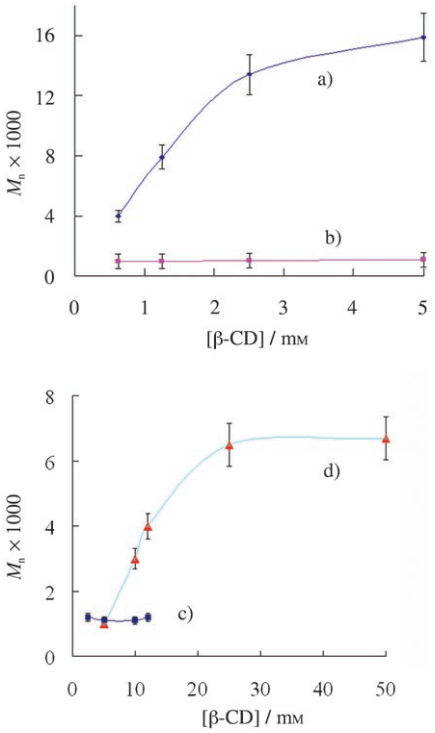


Figure 8. Effect of concentration of the modified β-CD on the molecular weight of a) **3**, b) α-CD, c) **4**, and d) **2** observed by VPO at 40 °C.

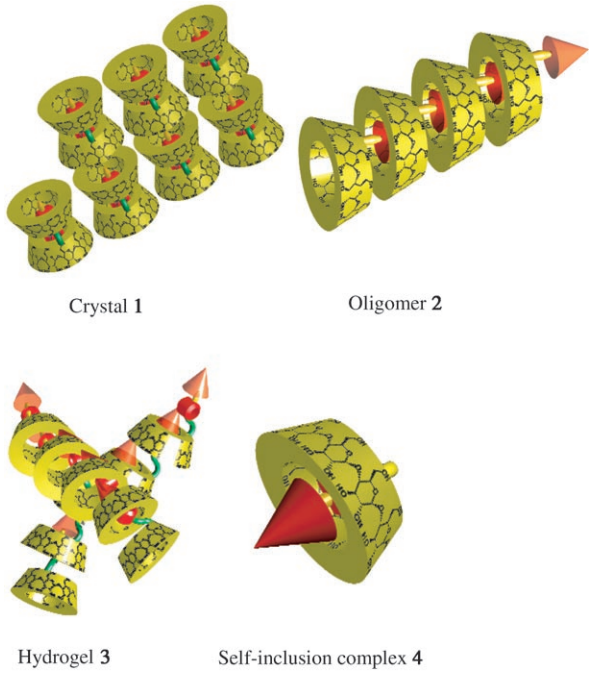


Figure 9. Schematic representation of assemblies of compounds **1**–**4**.

One of main purposes of this research is to develop a better understanding of supramolecular gelation. Therefore, we attempted to observe the gelation process directly by AFM (Figure 10). Samples of different concentrations were prepared on the mica substrate by slow evaporation of water at room temperature overnight. At a concentration of 5×10^{-8} M (Figure 10a), one-dimensional fibers were observed; this is the initial step for gelation and implies that gelation begins with supramolecular polymer formation through host–guest interactions. Increasing the concentration twofold ramified the fibrils into a grid (Figure 10b). Figure 10b shows that the fibrils are composed of monofibrils and bundles, and that the aggregation between the fibrils is attributed to hydrogen bonding between CDs. The results suggest that the hydrogen bonds between β-CD molecules on the neighboring supramolecular polymer chains act as physical cross-links in the gel network. The sample with a concentration of 5 mM produced the structure in Figure 10c, which is a typical image of a gel made up of small organic molecules. Numerous fibrils of compound **3** were observed, and their height was much larger than that of the CD ring due to the entanglement of several fibrils. All the fibrils cross-linked with each other to form a three-dimensional network. Hydrogen bonds between neighboring fibrils are proposed as physical cross-linkers owing to the abundance of OH groups in the CD ring. Therefore, the proposed mechanism of supramolecular gelation is as follows:

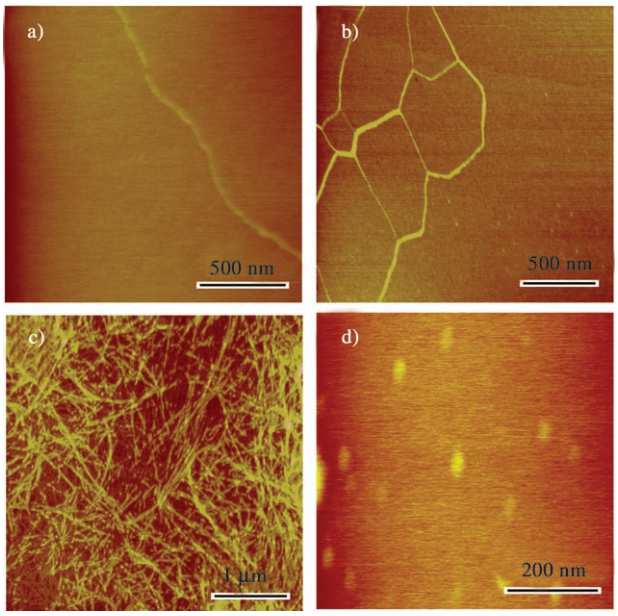


Figure 10. AFM images of **3**. Samples were acquired from concentrated aqueous solution on mica substrate. The concentrations of the solutions were a) 5×10^{-8}, b) 1×10^{-7}, and c) 5×10^{-3} M of **3**, and d) 5×10^{-8} M of native β-CD.

initially, supramolecular fibrils of the modified CD form by self-assembly through host–guest interactions. The elongated fibrils are then cross-linked through hydrogen bonds between CDs to form the hydrogel.

Chemical-Responsive Properties of 3

Next, we focused our interest on the chemical-responsive properties of the supramolecular hydrogel by adding a competitive guest, host, and denaturing compound.

Figure 11 shows photographs for the gel-to-sol transitions. A gel-to-sol transition occurred either by the addition of 2 equivalents of AdCA or 2 M urea as a strong guest compound or denaturing reagent. The chemical-responsive properties of **3** were monitored by ^{1}H NMR and induced circular dichroism (ICD) spectra. The observed ICD band in the 220–350-nm wavelength regions can be assigned to the circular dichroism band induced by the inclusion of guest parts in β-CD. The partial ^{1}H NMR spectrum of **3** in the presence of AdCA revealed that the signals of the guest proton shifted downfield. The dispersed peaks of the C1–H protons shifted to around 5.1 ppm. Furthermore, the ICD spectrum of **3** with AdCA showed that the peak intensities were significantly weakened at wavelengths of around 233, 259, and 313 nm, and positive-to-negative changes occurred at wavelengths of around 282 and 353 nm. These changes suggest that the guest parts are expelled from the CD cavities by AdCA and break the supramolecular polymers.

Upon treatment of hydrogel **3** with 2 M urea, the characteristic signals in both the ^{1}H NMR and the ICD spectra did not reveal changes, which implies that the guest parts remained within the CD cavity. It is known that urea acts as a denaturing agent that breaks the hydrogen bond.[13] Thus, the gel-to-sol transition occurs because urea breaks the hydrogen bond between CDs that serve as cross-linkers, which makes the three-dimensional network in the hydrogel collapse. These results agree with the proposed mechanism.

Upon the addition of β-CD, a precipitate was obtained instead of hydrogel. The ^{1}H NMR spectrum of **3** did not show a change. However, the ICD bands at 220–250 and 295–340 nm decreased as the β-CD concentrations increased (see Supporting Information). Therefore, the destroyed hydrogel in the presence of β-CD should be ascribed to the native β-CD included the guest parts, which act as competitive hosts to shorten the fibrils.

Furthermore, the reversible chemical-responsive gel–sol transitions were investigated. It was reported that the association constant for 1:1 complex formation of methyl orange (MO) with β-CD ($851\,M^{-1}$) is larger than that with α-CD ($229\,M^{-1}$).[14] Therefore, MO and α-CD were chosen as the competitive guest and host, respectively. Gel-to-sol and sol-

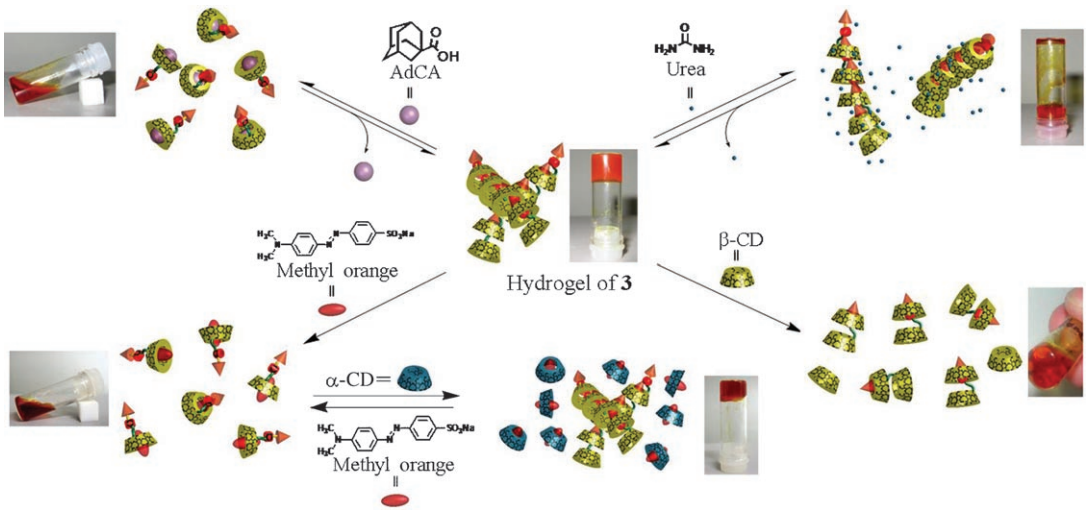


Figure 11. Conceptual illustration and photographs of the chemical-responsive supramolecular hydrogel **3**.

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to-gel transitions occurred after the alternate addition of MO and α-CD. The reversible transition occurred four times until solubility was reached to the limit. This procedure was monitored by ^{1}H NMR spectroscopy (Figure 12). When 1.0 equivalent of MO was added to 5 mM **3**, all the peaks of the guest protons were split. The new peaks from protons e, b, d, and a shifted to higher field, which agrees with the ^{1}H NMR spectral changes in the presence of AdCA, and are indicative of partial guests being released from the β-CD cavity. When 2.0 equivalents of MO were added, more than 60% of the guest parts were released, as calculated from the peak of the TNB group. Next, α-CD was added to the mixture as a competitive host. Shifts to the original position were observed upon addition of α-CD. Over 85% of guests were again included in the α-CD cavity until 2 equivalents of α-CD was added. It is known that the TNB group is too large to be included in α-CD. The peak in the spectrum of MO with α-CD in D_2O confirms that MO was included in the α-CD cavity and the guest of **3** was almost included in β-CD. Therefore, the mechanism can be explained as follows: MO acts as a competitive guest to destroy the supramolecular fibrils, which leads to the gel-to-sol transition. Then, MO is withdrawn from the β-CD cavity by α-CD due to the higher affinity, which allows the gel to reform.

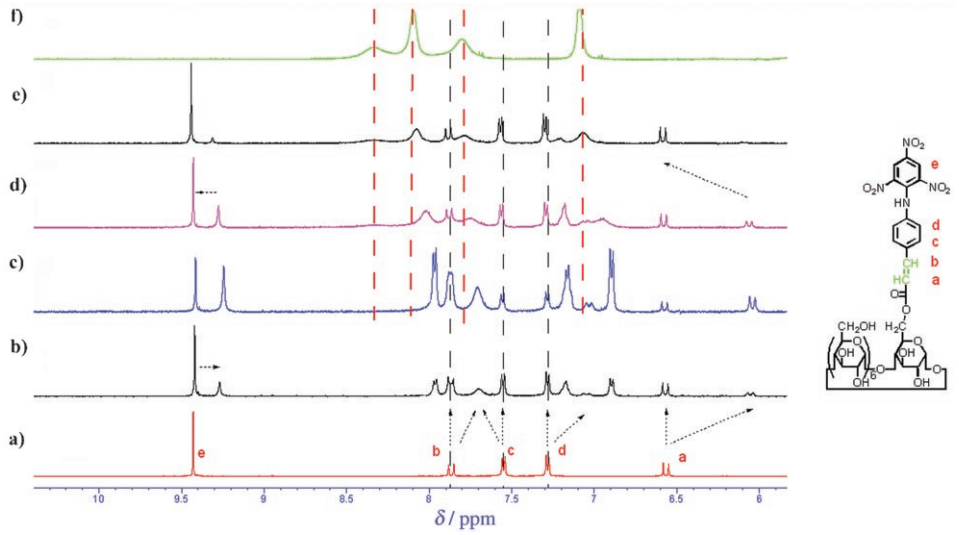

Figure 12. ^{1}H NMR spectra (500 MHz) of a) **3** (5 mM), b) **3**+1.0 equivalent MO, c) **3**+2.0 equivalents MO, d) **3**+2.0 equivalents MO+1.0 equivalent α-CD, e) **3**+2.0 equivalents MO+2.0 equivalents α-CD, and f) MO (10 mM)+α-CD (10 mM) in D_2O at 30 °C.

Conclusions

We have presented a novel method of preparing a supramolecular hydrogel from modified cyclodextrin without a polymer backbone. The present method is based on the host–guest and hydrogen-bonding interactions of CDs. The host–guest interactions contribute to supramolecular polymer formation as fibrils, whereas the hydrogen bonds act as cross-linkers. Initially, compound **2** was synthesized to confirm the role of the TNB group, and it was found by PFG NMR and AFM that compound **2** forms a supramolecular polymer in aqueous solution. These observations imply that modified β-CD with a TNB group at the 6-position formed favorably in a tail-to-head manner for β-CD in aqueous solution. Then, the TNB group was linked to compound **1** to give compound **3**. The novel modified β-CD forms supramolecular fibrils through host–guest interactions, whereas hydrogen bonds between the cross-linked fibrils of the CDs give a hydrogel. The hydrogel showed excellent chemical-responsive properties. Gel-to-sol transitions occurred by adding AdCA as a competitive guest or urea as a denaturing reagent, and the hydrogel was destroyed by native β-CD as a competitive host. Additionally, the gel-to-sol transition occurred upon addition of MO as a guest compound, whereas the reverse reaction occurred upon addition of α-CD, which is a stronger host for MO. The reversible gel–sol transitions occurred four times upon the alternate addition of MO and α-CD.

Experimental Section

Measurements

^{1}H NMR spectra were recorded on a 400 MHz JEOL JNM EX-270 spectrometer at 30 °C. Chemical shifts were referenced to the internal standard in the solvent. 2D ROESY NMR and PFG NMR experiments were performed in D_2O at 30 °C at 500 MHz on a JEOL JNM LA-500 NMR spectrometer and at 600 MHz on a VARIAN UNITY PLUS-600 NMR spectrometer, respectively. The WETBPPSTE pulse sequence was applied for PFG NMR spectroscopy,[10] and the pulsed field gradients were increased from 0.3 to 20.7 Gauss cm^{-1}. The time separations between the pulsed field gradients and the duration for which they were applied were 100 and 1.1 ms. FTIR spectroscopy was performed on a Jasco FT/IR-410 spectrometer. KBr was used as the dispersant. Elemental analysis was performed on an Elementar Vario EL-III instrument. Circular dichroism and UV/Vis spectra were recorded on a Jasco J820 spectrometer in water with a 0.1-cm cell at room temperature. Positive-ion matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry experiments were performed on a Shimadzu/Kratos Axima CFR V2.2.1 mass spectrometer. Vapor pressure osmometry measurements were performed on a Knauer No. A0280 vapor osmometer at 40 °C. Aqueous NaCl and α-CD were used as the instrument standard. AFM images were obtained by using a Nanoscope IIIa instrument (Digital Instruments, Santa Barbara, CA). The samples were prepared by slow evapora-

tion of a dilute aqueous solution of the CD derivatives on mica overnight.

Materials

β-CD, sodium hydroxide (NaOH), and *p*-toluenesulfonyl chloride were obtained from Nacalai Tesque, Inc. *trans*-4-Aminocinnamic acid, *trans*-4-aminohydrocinnamic acid, sodium 2,4,6-trinitrobenzene-1-sulfonate dehydrate (TNBS), and AdCA were obtained from Tokyo Chemical Industry Co., Ltd. [D_6]DMSO (DMSO = dimethyl sulfoxide) and D_2O were purchased from Aldrich.

Syntheses

6-Amino-β-CD and **1** (βCD-CiAm): These compounds were prepared according to the method reported previously.[9]

2 (βCD-AmTNB): TNBS (390 mg, 1.11 mmol) was added to a solution of 6-amino-β-CD (486 mg, 0.5 mmol) in H_2O (20 mL), and the mixture was stirred for 1 day. The obtained orange transparent solution was poured into acetone. The precipitate was filtered, washed with acetone, and dried under vacuum. Yield: 75%. ^{1}H NMR (400 MHz. [D_6]DMSO): δ = 8.95 (br s, 2H, TNB), 5.81–5.61 (m, 14H, O2 and O3 of β-CD), 4.94–4.79 (m, 7H, C1 of β-CD), 4.50–4.25 (m, O6 and C6′ of β-CD), 3.92–3.16 ppm (m, overlapped with HOD); MS (MALDI-TOF): *m*/*z* calcd for $C_{48}H_{74}O_{40}N_4$: 1371 $[M+Na]^+$; found: 1370.4.

3 (βCD-CiAmTNB): AdCA (35.2 mg, 0.195 mmol) was added to a suspension of **1** (83.2 mg, 0.0648 mmol) in H_2O (30 mL). After the mixture was stirred for 12 h at room temperature, the suspension gradually became clear, and the precipitate was removed by filtration. Then, TNBS (39.0 mg, 0.111 mmol) was added, and the mixture was stirred for one more day. The obtained orange transparent solution was poured into acetone. The precipitate was filtered, washed with acetone, and dried under vacuum. Yield: 62%. IR: $\tilde{\nu}$ = 3380, 2928, 1636, 1514, 1345, 1031 cm^{-1}; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 10.22 (s, 1H, NH), 8.81 (br s, 2H, TNB), 7.63–7.56 (m, 3H, phenyl and CH=), 7.05 (br s, 2H, phenyl), 6.59 (d, J = 16.1 Hz, 2H, CH=), 5.77–5.64 (m, 14H, O2 and O3 of β-CD), 4.90–4.83 (m, 7H, C1 of β-CD), 4.50–4.25 (m, O6 and C6′ of β-CD), 3.92–3.16 ppm (m, overlapped with HOD); ^{13}C NMR (400 MHz, [D_6]DMSO): δ = 166.0 (CO), 139.8, 139.6, 129.4, 129.4, 126.6, 126.6, 126.5 (phenyl), 101.9 (C1 of β-CD), 81.6 (C4 of β-CD), 73.0 (C3 of β-CD), 72.4 (C2 of β-CD), 72.0 (C5 of β-CD), 59.9 (C6 of β-CD), 58.7 ppm (C6′ of β-CD); MS (MALDI-TOF): *m*/*z* calcd for $C_{57}H_{78}O_{42}N_4$: 1514 $[M+Na]^+$; found: 1515.1; elemental analysis: calcd (%) for $C_{57}H_{78}O_{42}N_4 \cdot 8\,H_2O$: C 41.86, H 5.79, N 3.43; found: C 42.01, H 5.88, N 3.31.

4 (βCD-HyCiAmTNB): TNBS (80.0 mg, 0.222 mmol) was added to a suspension of 6-AmHyCiO-β-CD (166 mg, 0.13 mmol) in H_2O (10 mL). After the mixture was stirred for 1 day, the suspension gradually became clear. The solution was concentrated to 5 mL and poured into acetone (50 mL). The resulting precipitate was washed three times with acetone and dried under vacuum to give the crude product. Yield: 65%. IR: $\tilde{\nu}$ = 3375, 2931, 1719, 1621, 1339, 1029 cm^{-1}; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 10.16 (br s, 1H, CONH), 8.83 (br s, 2H, TNB), 7.16 (d, J = 7.8 Hz, 2H, phenyl), 6.98 (br s, 2H, phenyl), 5.77–5.64 (m, 14H, O2 and O3 of β-CD), 4.85–4.82 (m, 7H, C1 of β-CD), 4.44–4.28 (m, O6 and C6′ of β-CD), 3.84–3.16 (m, overlapped with HOD), 2.82 (t, J = 7.3 Hz, 2H, CH_2), 2.67–2.60 ppm (m, 2H, CH_2); ^{13}C NMR (400 MHz, [D_6]DMSO): δ = 171.8 (CO), 140.3, 138.7, 135.0, 129.2, 128.9, 127.6, 126.8, 121.1 (phenyl), 101.9 (C1 of β-CD), 81.6 (C4 of β-CD), 73.0 (C3 of β-CD), 72.4 (C2 of β-CD), 72.0 (C5 of β-CD), 59.9 (C6 of β-CD), 57.2 (C6′ of β-CD), 34.6 (CH_2), 29.6 ppm (CH_2); MS (MALDI-TOF): *m*/*z* calcd for $C_{57}H_{80}O_{42}N_4$: 1516 $[M+Na]^+$; found: 1518.6; elemental analysis: calcd (%) for $C_{57}H_{80}O_{42}N_4 \cdot 7.5\,H_2O$: C 42.04, H 5.88, N 3.44; found: C 42.10, H 5.83, N 3.39.

6 (NaCiTNB): NaOH (10 mg, 0.25 mmol) was added to a suspension of 4-aminohydrocinnamic acid (32 mg, 0.20 mmol) in H_2O (10 mL). TNBS (80.0 mg, 0.222 mmol) was added, and the solution was stirred for 3 h at 90 °C. After the mixture was cooled to room temperature, HCl (10 M, 0.5 mL) was added to the solution. The resulting precipitate was washed three times with water and dried under vacuum. Yield: 89%. IR: $\tilde{\nu}$ = 3272, 3085, 1690, 1628, 1511, 1345 cm^{-1}; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 10.23 (s, 1H, CONH), 8.96 (s, 2H, TNB), 7.63 (d, J = 8.5 Hz, 2H, phenyl), 7.53 (d, J = 16.2 Hz, 1H, CH=), 7.17 (d, J = 8.6 Hz, 2H, phenyl), 6.48 ppm (d, J = 15.9 Hz, 1H, CH=); ^{13}C NMR (400 MHz, [D_6]DMSO): δ = 167.3 (CO), 142.7, 118.5 (CH=CH), 140.1, 139.8, 136.8, 136.4, 131.1, 129.1, 126.6, 120.3 ppm (phenyl); elemental analysis: calcd (%) for $C_{15}H_{10}N_4O_8$: C 48.14, H 2.69, N 14.97; found: C 48.15, H 2.74, N 15.11.

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