Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

pH-Responsive Phase Transition of Supramolecular Hydrogel Consisting of Glycosylated Amino Acetate and Carboxylic Acid Derivative

Shigeki Kiyonaka[a]; Shan-Lai Zhou[a]; Itaru Hamachi[b]
[a] Department of Chemistry and Biochemistry, Kyushu University, Fukuoka, Japan [b] Institute for Fundamental Research of Organic Chemistry (IFOC), Kyushu University, Fukuoka, Japan

Online publication date: 13 May 2010

To cite this Article Kiyonaka, Shigeki , Zhou, Shan-Lai and Hamachi, Itaru(2003) 'pH-Responsive Phase Transition of Supramolecular Hydrogel Consisting of Glycosylated Amino Acetate and Carboxylic Acid Derivative', Supramolecular Chemistry, 15: 7, 521 — 528
To link to this Article: DOI: 10.1080/1061027031000160511 5
URL: http://dx.doi.org/10.1080/10610270310001605115

Supramolecular Chemistry, 2003 Vol. 15 (7–8), pp. 521–528

pH-Responsive Phase Transition of Supramolecular Hydrogel Consisting of Glycosylated Amino Acetate and Carboxylic Acid Derivative

SHIGEKI KIYONAKA[a], SHAN-LAI ZHOU[a] and ITARU HAMACHI[a,b,c,*]

[a]*Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka, 812-8581, Japan;* [b]*PRESTO (Organization and Function, JST), Fukuoka, 812-8581, Japan;* [c]*Institute for Fundamental Research of Organic Chemistry (IFOC), Kyushu University, Fukuoka, 812-8581, Japan*

Received 2 November 2002; Accepted 10 January 2003

By addition of a carboxylated amino acetate (2) to a low-molecular-weight hydrogel (1) which has a unique thermally induced volume-phase transition character, a macroscopic pH-responsive feature is newly conferred on the supramolecular hydrogel. The direct observation of temperature-dependent behavior of the mixed hydrogel clearly showed that the thermally induced swelling-shrinkage type of the volume phase transition at pH 4 is shifted to the gel–sol transition at pH 7 by 10 mol% addition of 2 to the hydrogel 1. On the basis of the measurements by TEM, SEM, XRD and FT-IR, it is conceivable that incorporation of the anionic carboxylate of 2 slightly disturbs the packing of the hydrogen bond belt of the mixed hydrogel. Such a slight disturbance greatly leads to the sol–gel transition by elevating temperature, instead of the volume-phase transition. Introduction of dynamic characteristics to supramolecular systems in a macroscopic level may extend the potential of these materials in various fields.

Keywords: Hydrogel; Glycosylated amino acid; pH response; Supramolecular materials

The Future of Supramolecular Chemistry

One future for supramolecular chemistry lies in biology- and biomolecular science-oriented research, such as new artificial receptors, modulators for bio-macromolecules and novel matrices or materials which can regulate living cells. Application of supramolecular approaches to these topics is being actively pursued in the Hamachi group. This paper discusses a pH-responsive feature of the supramolecular copolymer hydrogel consisting of a glycosylated amino acetate and a structurally related carboxylic acid. This is important because we have demonstrated that the supramolecular concept provides a great synthetic accessibility to the intelligent materials. These macroscopic responses towards physiological environments such as pH, temperature and biological substances should extend the potential of supramolecular biomaterials.

Professor Itaru Hamachi obtained a PhD from Kyoto University, Japan in 1988 and was engaged as an assistant professor in Kyushu University (emeritus Professor Kunitake's group) at the same time. In 1992, he moved to Professor Shinkai's group as an associate professor and then was promoted to a full professor in IFOC, Kyushu University in 2001. He is also now a PRESTO investigator (JST). His research interests are in the range of bio-organic and bio-inorganic chemistry, protein engineering, chemical biology and supramolecular chemistry.

*Corresponding author. E-mail: itarutcm@mbox.nc.kyushu-u.ac.jp

ISSN 1061-0278 print/ISSN 1029-0478 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1061027031000160511 5

INTRODUCTION

Various functional molecules have emerged from supramolecular chemistry, such as sophisticated chemosensors [1], molecular switches or logic gates [2,3], molecular machines [4–6], organic zeolites [7], molecular magnets [8], and supramolecular polymers [9]. In the field of materials science, supramolecular polymers are especially attractive because of their unique properties [10–12]. Since these polymers are formed by the association of the monomeric components through unidirectional noncovalent interactions, these are anticipated to be advantageous over traditional polymers in several points of view. The most attractive feature is their reversible formation and de-formation of polymer due to their noncovalent self-assembly and disassembly process. It is generally considered that the dynamic property is essential for the development of molecular-based intelligent materials in the next generation. Because of the high flexibility of supramolecular polymers in their molecular design, a variety of dynamic characteristics have been readily introduced, and these may extend the potential application to intelligent materials.

In the field of supramolecular polymeric materials, supramolecular organogels [13–16] and hydrogels [17–21] have been actively developed. These gels consist of low-molecular-weight gelators by a self-assembling manner to solidify many solvents. Structural analysis of these gels reveals that gel fibers are stabilized by various unidirectional intermolecular interactions so as to enforce one-dimensional self-assembly, like other supramolecular polymers. By using a combinatorial approach [22–24], we recently discovered supramolecular hydrogels based on glycosylated amino acetate derivatives (**1**), which show a macroscopic thermal response [25]. These unique supramolecular hydrogels reversibly swell and shrink, regardless of the non-polymeric material. Such a thermally induced volume response is almost the same as that of poly-NIPAM (*N*-isopropylacrylamide), which is well known as a thermally responsive conventional polymer [26]. In this paper, we find that the addition of a carboxylated amino acetate (**2**) to a hydrogelator (**1**) can confer a new pH-responsive property on the supramolecular hydrogel (Fig. 1). The thermally induced swelling-shrinkage type of volume phase transition at pH 4 is shifted to the gel–sol transition at pH 7 by addition of 10 mol% **2** to the hydrogel **1**.

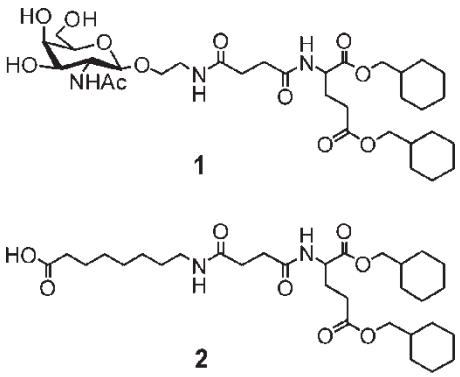

FIGURE 1 Structure of glycosylated amino acetate **1** and carboxylated amino acetate **2**.

RESULTS AND DISCUSSION

Glycosylated amino acetate (**1**) consists of three components (glycosyl, succinamide, and glutamate modules). A carboxylated amino acetate (**2**) was employed as a pH-responsive additional monomer. The additive (**2**), having the succinamide and glutamate modules that are identical to the modules of the corresponding glycosylated amino acetate (**1**), was designed to show the excellent miscibility. The methylene chain length of the carboxylate module was designed to be slightly longer than the molecular length of the glycosylated module, which is expected to expose the carboxylate group to the hydrophilic interface of gel fibers. The new supramolecular hydrogel comprising **1** and **2** was obtained with simple mixing and heating, followed by aging at 25°C.

As reported previously, the hydrogel consisting of **1** displayed temperature-induced shrinkage. Interestingly, the thermally induced phase-transition behavior changed depending on the pH condition when 10 mol% of **2** was mixed with the hydrogelator **1**. Figure 2 shows photographs of the mixed hydrogel at different pHs at high and low

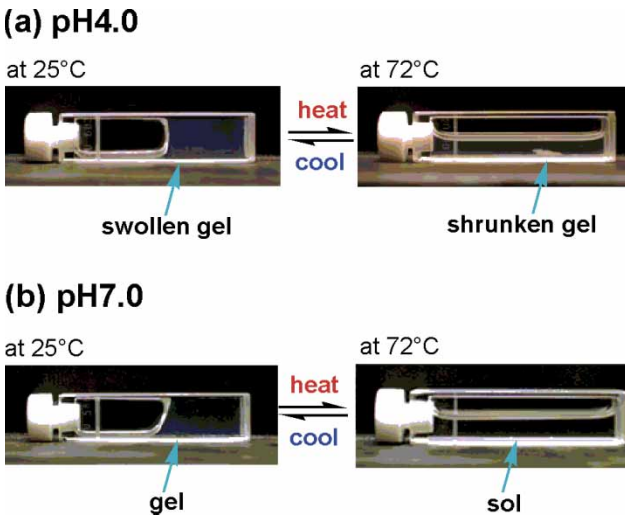

FIGURE 2 Photograph of the hydrogel **1** mixing with **2** at (a) pH 4.0 and (b) pH 7.0. [amino acetates] = 0.5 wt%, **1** : **2** = 10 : 1. (See colour plate 1 at the end of this issue.)

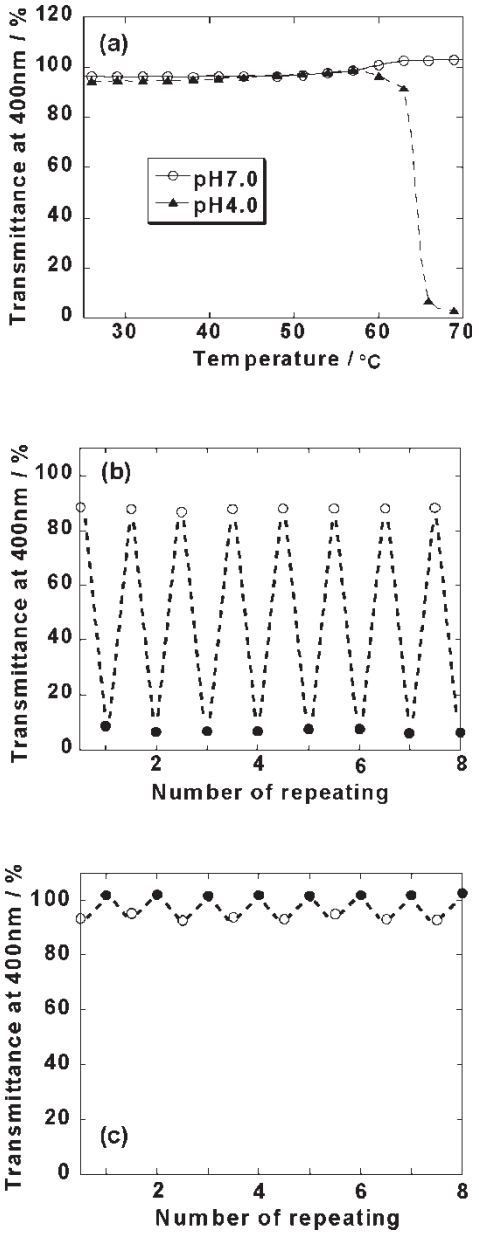

FIGURE 3 Transmittance change in the mixed hydrogel depending on (a) temperature, and pH: (b) at pH 4 and (c) pH 7. [amino acetates] = 0.5 wt%, **1** : **2** = 10 : 1.

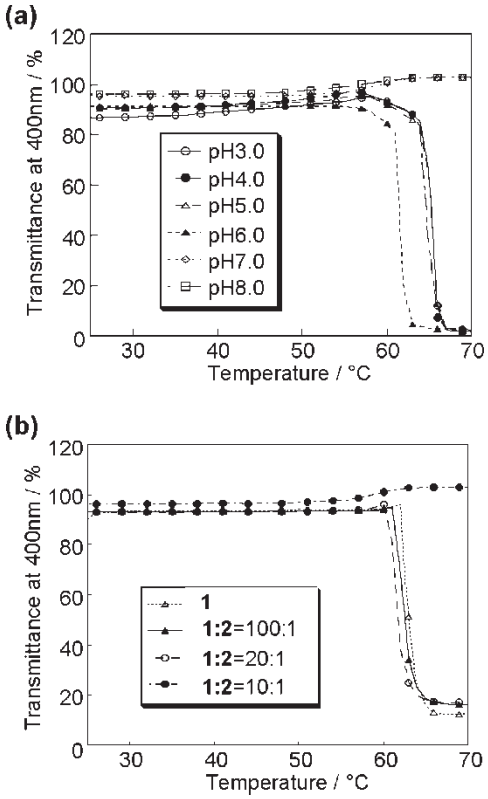

FIGURE 4 Thermally induced transmittance change in mixed hydrogel depending on (a) pH and (b) the content ratio of **2**. [amino acetates] = 0.5 wt%.

temperatures. At pH 4, a swollen hydrogel formed at room temperature, and this gel shrank with an increase in temperature, similar to the single-component hydrogel of **1**. In contrast, the swollen gel at room temperature completely melted into a homogeneous solution at high temperatures at pH 7 instead of shrinking. That is, a typical gel–sol transition was observed at pH 7.

These processes can be monitored spectroscopically using the transmittance change in each state of the hydrogel. The precipitates formed from the shrunken gel cause the light scattering, and as a result, the transmittance of visible light greatly decreases during gel shrinkage. However, the melted hydrogel (i.e. sol state) gives a clear solution so that the transmittance slightly increases relative to that in the gel state. Figure 3a shows a typical example of temperature-dependent transmittance change in the above-mentioned hydrogel. Clearly, the transmittance of the gel at pH 4 dramatically decreases at 65°C, which reflects the gel shrinkage. However, in the case of the hydrogel at pH 7, the transmittance slightly increases at 65°C, which corresponds with the gel–sol transition behavior as shown in Fig. 2. These phase-transition processes are reversible, so they can be repeated many times by simply increasing or decreasing the temperature, as shown in Fig. 3b and c. In the case of volume transition at pH 4, the transmittance drastically decreases at 70°C and recovers at 25°C. In the case of the gel–sol transition at pH 7, the transmittance slightly increases at 70°C and decreases at 25°C.

A switching of the phase transition behavior occurs in the sharp pH range. Figure 4a shows the transmittance change in a mixed gel between pH 3.0 and 8.0. Below pH 6, the temperature-dependent curves of transmittance show a typical volume phase transition, whereas the curve changes to a gel–sol transition above pH 7. The content of the additive **2** also affects switching of the phase transition. This can be seen by the transmittance change at pH 7 (Fig. 4b). At less than 5 mol% of **2**, the mixed gel shows the volume phase transition, like the **1** gel

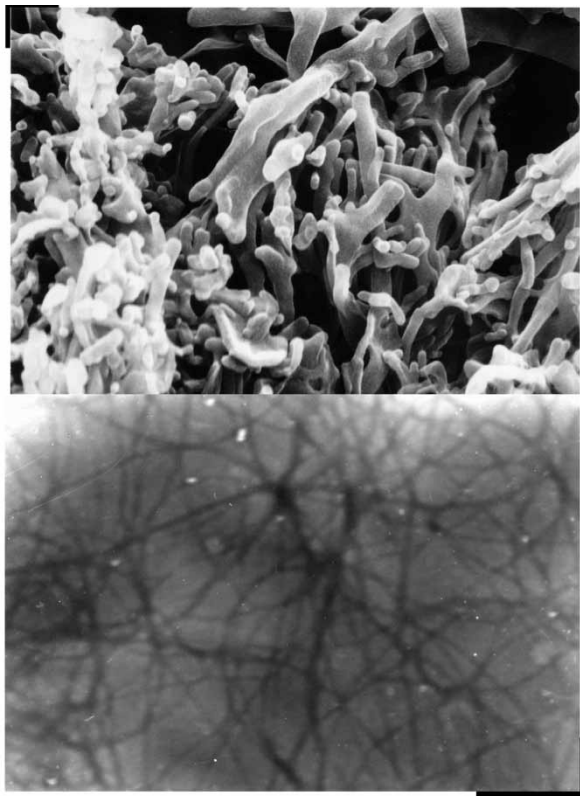

FIGURE 5 SEM observation of the xerogel of the mixed hydrogel coated by Pt (a), and TEM photograph of the mixed hydrogel without staining (b).

itself. The sol–gel phase transition is observed in the mixed gel bearing 10 mol% of **2**. These results clearly indicate that the carboxylate–carboxylic acid equilibrium in **2** controls the phase-transition property of the hydrogel.

The microstructure of the gel was inspected using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray diffraction (XRD) measurements. Using SEM, fibrous structures with a diameter of several hundred nanometres could be observed in the freeze-dried sample (xerogel) of mixed hydrogel (Fig. 5a). TEM revealed many entangled fibers of mixed gel with a higher resolution when the gel had dried on a grid (Fig. 5b). The contrast against the bulk space was obtained without staining, and the diameter of the fibers could be estimated to range from 300 to 1000 nm. This morphology is similar to that of the hydrogel consisting of the single component **1**. These data suggest that small molecules assemble non-covalently into long fibers, from which many cavities form so that water molecules can be fixed. It is interesting that XRD data of the xerogel revealed a strong diffraction peak at 3.8 nm, clearly suggesting the regular structure of the gel fibers, which may be attributed to the tilted bimolecule unit of the gelator. Since the gel formed in water, it is reasonable that the bimolecular aggregate assembled at the hydrophobic methylcyclohexyl moiety and the GalNAc unit exposed to the water phase. Two broad peaks corresponding to 0.44 nm were also observed, in good agreement with the saccharide or cyclohexyl ring thickness. These may be ascribed to the tight packing of the **1a**. The TEM photo and XRD revealed no significant differences in the pattern in the gel at pH 3.5 and pH 7.4, indicating that the fundamental structure of the gel is almost the same at both pHs.

Distinct differences between these mixed hydrogels prepared in acidic or neutral conditions were detected by FT-IR measurement. We previously reported a well-developed hydrogen-bonding network through the succinamide moiety of **1** in a single-component hydrogel [25]. In the mixed hydrogel at pH 4, the greatly shifted IR peak was mainly observed at around 1614 cm^{-1} due to

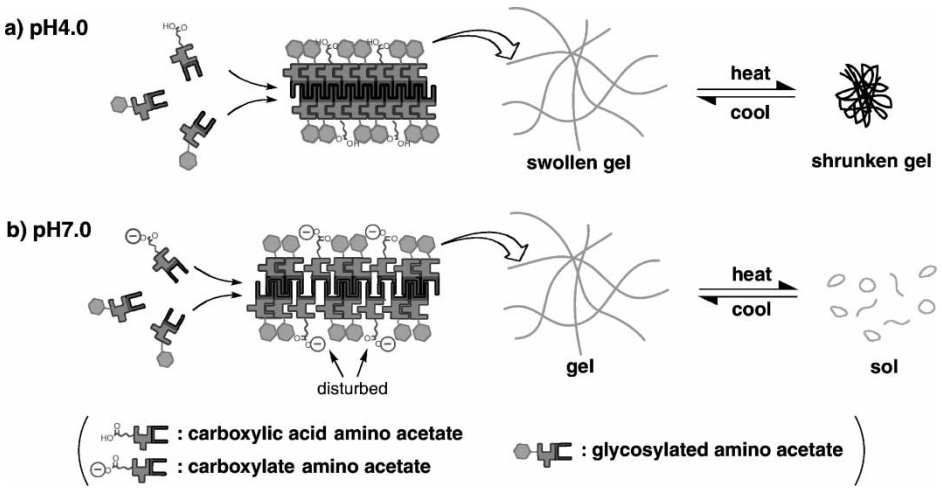

FIGURE 6 Schematic illustration of the present pH-responsive supramolecular hydrogel.

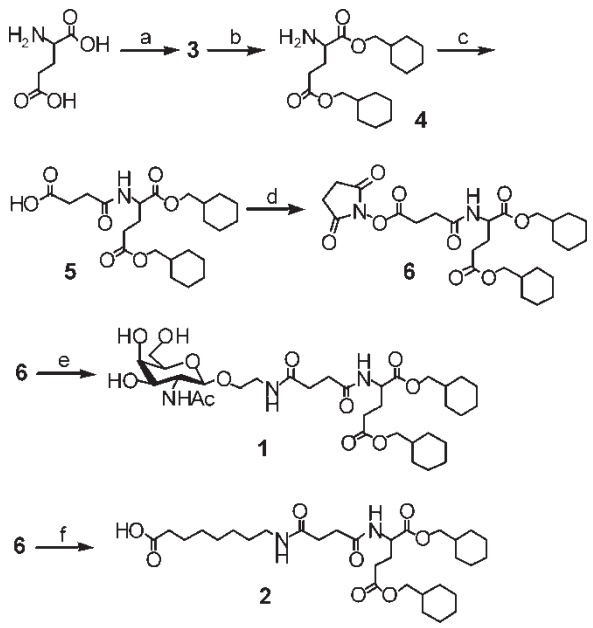

FIGURE 7 Synthetic route of glycosylated amino acetates and carboxylated amino acetate. Reagents and conditions: (a) cyclohexyl methanol, $TsOH{\cdot}H_2O$/toluene; (b) Amberlite IRA96SB/CH_2Cl_2; (c) succinic anhydride, DIEA/dry CH_2Cl_2; (d) *N*-hydroxy succinimide, EDC/dry DMF; (e) 7/dry DMF, dry pyridine; (f) 8-aminooctanoic acid/dry DMF, dry pyridine.

the amide carbonyl stretching, similar to the previous data. However, the corresponding peak shifted to 1624 cm^{-1} in the gel prepared under pH 7.0, indicating that the hydrogen bonding is weakened under neutral conditions. It is noteworthy that the IR peak resulting from the carbonyl stretching shifted to 1632 cm^{-1} in the sol state by heating, and the fibrous structure disappeared in the TEM observation. However, the carbonyl peak did not move significantly in the shrunken gel at pH 4. The ^{1}H-NMR spectrum of the precipitated shrunken gel showed that the ratio of **1** to **2** was 100:19, which is roughly in agreement with the mixed ratio when the gel was prepared. This implies that **2** is homogeneously mixed with the gel fiber of **1**.

From the above data, we may illustrate the present supramolecular hydrogel as follows (see Fig. 6): monomers **1** and **2** are mixed and self-assembled into fibers in a broad pH range. When the head group of **2** changes to the anionic carboxylate under a neutral pH, the anions generated slightly disturb the well-developed packing of the hydrogen bond belt of the mixed hydrogel, relative to that under acidic pH. At room temperature, such a disturbance does not affect the gel morphology. However, by elevating the temperature, the less well-ordered assembly cannot maintain the fibrous structure, thus inducing the sol state instead of the gel shrinkage.

CONCLUSION

This paper shows that a new additive to the non-ionic supramolecular hydrogel successfully modulates the thermal phase-transition behavior in response to the environmental pH conditions. At an acidic pH, the mixed hydrogels showed a swelling-shrinkage type of volume transition, whereas they showed a typical gel–sol transition at a neutral pH. Such a pH response is mainly attributed to the acid–base property of the newly added carboxylate-terminated amino acetate (**2**).

In the research field of polymer science, poly-NIPAM is widely used as a typical thermally responsive polymer, and other stimuli-responsive hydrogels have been produced by copolymerization of an additional monomer with poly-NIPAM for a wide range of applications such as artificial muscles, artificial valves, controlled drug delivery or release materials [27–30], and so on. In supramolecular polymers, in contrast, suitable materials displaying a macroscopic volume response have not been reported so far, and our recent results imply that the present glycosylated amino acetate is one of the promising structures comparable with poly-NIPAM. We have demonstrated in this paper that the coplymerization approach is applicable to supramolecular polymers, which may facilitate in the development of various stimuli-responsive supramolecular hydrogels. It is beneficial that a new component is readily incorporated into the main framework in the case of supramolecular hydrogel by simple mixing to provide a supramolecular co-polymer. Furthermore, these components are easily recyclable because of non-covalent association. Using this methodology, one can envisage various types of supramolecular hydrogels bearing the macroscopic stimuli-responsible property. My group is now working in this field.

EXPERIMENTAL

A synthetic scheme of the amino acetate derivatives is shown in Fig. 7. Experimental details are described below.

Materials and Methods

All chemical reagents were obtained from Aldrich, Sigma, TCI, Wako, Organo, or Watanabe chemical industries. Commercially available reagents were used without further purification except for Amberilite IRA96SB. Amberlite IRA96SB was washed with MeOH and CH_2Cl_2 before use. Solvents were dried according to the standard procedure. ^{1}H-NMR spectra were obtained on a JOEL JNM-EX400 (400 MHz). Mass spectra were

recorded on a MALDI-TOF-Mass spectrometer (PE Biosystems Voyager DE-RP).

Synthesis of Di-methyl-cyc-hexyl Glutamate 4

L-Glutamic acid 7.18 g (48.8 mmol) was suspended in 100 ml of toluene, and 15.6 ml (127 mmol) of cyclohexyl methanol, and 12.1 g (63.4 mmol) of *p*-toluenesulfonic acid monohydrate ($TsOH{\cdot}H_2O$) were added. A reflux condenser and Dean–Stark trap were attached to the reaction flask, and the reaction mixture was then refluxed overnight with dehydration. The reaction mixture was cooled and stored at 4°C overnight. Crystallized white solid **3** was obtained after collection and drying. Yield: 19.4 g (78%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 8.34 [br, 3H, COCH(NH_3^+)CH_2CH_2CO], 7.75 (d, 2H, $J = 8.4$, Ar–H), 7.13 (d, 2H, $J = 8.0$, Ar–H), 4.15 [m, 1H, CO*CH*(NH_3^+)CH_2CH_2CO], 3.91–3.80 (m, 4H, $COOCH_2C_6H_{11}$), 2.52–2.22 [m, 4H, COCH(NH_3^+)CH_2CH_2CO], 2.25 (s, 3H, Ar–CH_3), 1.80–0.85 (m, 22H, $COOCH_2C_6H_{11}$).

3 was dissolved in 500 ml of CH_2Cl_2, and 80 g of Amberlite IRA96SB (weak anion-exchange resin) were added. The mixture was then stirred for 1 h at room temperature. The anion-exchange resin was removed by suction filtration, and the filtrate was concentrated. After drying *in vacuo*, colorless oil **4** was obtained. Yield: 10.5 g (96%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 3.97–3.80 (m, 4H, $COOCH_2C_6H_{11}$), 3.47 [m, 1H, CO*CH*(NH_2)CH_2CH_2CO], 2.47 [t, 2H, $J = 7.6$, COCH(NH)CH_2CH_2CO], 2.07, 1.83 [m, 2H, COCH(NH)CH_2CH_2CO], 1.80–0.92 (m, 22H, $COOCH_2C_6H_{11}$).

Synthesis of 5

10.5 g (33.7 mmol) of **4** were dissolved in 120 ml of dry CH_2Cl_2 under a N_2 atmosphere. After adding 6.75 g (67.4 mmol) of succinic anhydride and 7.64 ml (43.8 mmol) of *N*,*N*′-diisopropyl ethyl amine (DIEA), the reaction mixture was stirred overnight at room temperature. The mixture was cooled to 0°C, and 3.00 ml (excess) of ethylenediamine were added to decompose the remaining succinic anhydride. The mixture was stirred for 1 h at room temperature and then diluted with 200 ml of CH_2Cl_2. The organic layer was washed with 150 ml of 1 N HCl × 6 and 100 ml of H_2O × 2, and dried over $MgSO_4$. After removing $MgSO_4$, solvent was removed. White solid **5** was obtained after drying *in vacuo*. Yield: 12.5 g (90%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 6.65 [d, 1H, $J = 8.0$, COCH(*NH*)CH_2CH_2CO], 4.62 [m, 1H, CO*CH*(NH)CH_2CH_2CO], 3.95, 3.89 (d, 4H, $J = 6.4$, 6.8, $COOCH_2C_6H_{11}$), 2.73–2.54 (m, 4H, COCH_2CH_2CO), 2.41 [m, 2H, COCH(NH)CH_2 CH_2CO], 2.22, 2.00 [m, 2H, COCH(NH)CH_2CH_2CO], 1.78–0.91 (m, 22H, $COOCH_2C_6H_{11}$).

Synthesis of NHS Activated Ester 6

5.00 g (12.2 mmol) of **3** and 2.11 g (18.3 mmol) of *N*-hydroxysuccinimide were dissolved in 60 ml of dry DMF under a N_2 atmosphere. 3.51 g (18.3 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were added into the solution at 0°C, and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with AcOEt/hexane (1/1, 400 ml), and the organic layer was washed with 100 ml of 0.5 N HCl × 5 and 100 ml of brine × 2. The organic layer was then dried and concentrated. Colorless oil **6** was obtained after drying *in vacuo*. Yield: 6.06 g (98%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 6.44 [d, 1H, $J = 8.0$, COCH(*NH*)CH_2CH_2CO], 4.63 [m, 1H, CO*CH*(NH)CH_2CH_2CO], 3.95, 3.88 (d, 4H, $J = 5.6$, 6.8, $COOCH_2C_6H_{11}$), 2.99 (m, 2H, $OCOCH_2CH_2CONH$), 2.83 [s, 4H, $COCH_2CH_2$-CO(succinamide)], 2.64 (m, 2H, $OCOCH_2CH_2$-CONH), 2.39 [m, 2H, COCH(NH)CH_2CH_2CO], 2.20, 1.99 [m, 2H, COCH(NH)CH_2CH_2CO], 1.78–0.91 (m, 22H, $COOCH_2C_6H_{11}$).

Synthesis of Glycosylated Amino Acetate 1 [*31*]

0.79 g (1.55 mmol) of **6** and 0.53 g (2.02 mmol, 1.3 eq) of aminoethyl-β-D-*N*-acetylgalactosamine **7** [32–34] were dissolved in 40 ml of dry DMF and 5 ml of dry pyridine under a N_2 atmosphere. The reaction mixture was stirred for 6 h at 40°C, and solvent was removed. The residue was purified by flush column chromatography (silica, $CHCl_3$: MeOH = 8 : 1 → 4 : 1) to obtain a white solid of glycosylated amino acetate **1**. Yield: 0.68 g (64%). ^{1}H-NMR (400 MHz, $CDCl_3$: CD_3OD = 5 : 1), δ7.80(d, 1H, $J = 7.8$, NH), δ7.60(t, 1H, $J = 5.9$, NH), δ4.50[m, 1H, CO*CH*(NH)CH_2CH_2CO], δ4.38(d, 1H, $J = 8.4$, H-1), δ3.97–3.51(m, 14H, H-2, H-3, H-4, H-5, H-6, OCH_2CH_2NH, $COOCH_2C_6H_{11}$), δ2.55–2.41[m, 6H, $COCH_2CH_2$CO, COCH(NH)CH_2 CH_2CO], δ2.15,1.90[m, 2H, COCH(NH)CH_2CH_2CO], δ2.03(s, 3H, CH_3CONH), δ1.74–0.96(m, 22H, $COOCH_2C_6H_{11}$). MALDI-TOF-Mass for $C_{33}H_{55}N_3O_{12}$ (MW = 685.80) : $m/z = 708.45$ $[M+Na]^+$, 724.34 $[M+K]^+$. Elemental anal. calcd for $C_{33}H_{55}N_3O_{12}{\cdot}1/2H_2O$: C, 57.04, H, 8.12, N, 6.05%. Found: C, 57.06, H, 8.02, N, 6.11%.

Synthesis of Carboxylated Amino Acetate 2

Carboxylated amino acetate **2** was obtained from **6** and 8-aminoctanoic acid according to the same method as that for **1**. The purification process was performed as follows. The residue was re-dissolved in 100 ml of $CHCl_3$, and the organic layer was washed with 100 ml of 1 N HCl × 5. The organic layer was dried and cocentrated. The residue was

purified by flush column chromatography (silica, $CHCl_3$: MeOH = 30 : 1 → 8 : 1) to obtain a white solid of carboxylated amino acetate **2**. Yield: 95%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 6.81 [d, 1H, $J = 7.6$, COCH(*NH*)CH_2CH_2CO], 6.12 (t, 1H, $J = 5.2$, CHNHCOCH_2CH_2CO*NH*CH_2), 4.59 [m, 1H, CO*CH*(NH)CH_2CH_2CO], 3.95, 3.89 (d, 4H, 6.4, 6.4, COO$CH_2C_6H_{11}$), 3.22 [m, 2H, NH$CH_2(CH_2)_5$ CH_2COOH], 2.60–2.32 [m, 8H, COCH_2CH_2CO, COCH(NH)CH_2CH_2CO, NH$CH_2(CH_2)_5CH_2$COOH], 2.22, 1.99 [m, 2H, COCH(NH)CH_2CH_2CO], 1.80–0.91 [m, 32H, NH$CH_2(CH_2)_5CH_2$COOH, COO$CH_2C_6H_{11}$]. MALDI-TOF-Mass for $C_{31}H_{52}N_2O_8$ (MW = 580.75) : $m/z = 603.75$ $[M + Na]^+$. Elemental anal. calcd for $C_{31}H_{52}N_2O_8$: C, 64.11, H, 9.02, N, 4.83%. Found: C, 64.11, H, 9.02, N, 4.79%.

Measurements

Preparation of Supramolecular Hydrogel

1. Preparation from buffer solution: A mixture (**1** : **2** = 10 : 1) of glycosylated amino acetate **1** and carboxylated amino acetate **2** was dissolved in methanol. The sample was dried *in vacuo* for 6 h and then suspended in buffer solution [25 mM acetate buffer (pH 3.0, 4.0, 5.0) or 25 mM phosphate buffer (pH 6.0, 7.0, 8.0)]. The suspension was heated for dispersion, and then the solution was subsequently cooled to room temperature. After aging at 25°C overnight, hydrogel was obtained.
2. Preparation from non-buffer solution: The dried sample was suspended in H_2O or D_2O, and then the suspension was dispersed by heating. HCl (DCl) solution or NaOH (NaOD) solution was added to adjust the pH (pD). The solution was re-heated for dispersion. After aging at 25°C overnight, hydrogel was obtained. The non-buffer sample was used for TEM and SEM observation, XRD measurement, and FT-IR measurement.

Observation of Gel Structure by Microscopy

A Hitachi H-600 electron microscope was used for TEM. A piece of the hydrogel was placed onto a carbon-coated copper grid and dried for 2 h under vacuum without staining. The grid was then examined with an accelerating voltage of 90 kV.

A Hitachi S-900 electron microscope was used for SEM. The hydrogel (5 ml) was frozen in liquid nitrogen. The frozen specimen was dried under vacuum for 24 h at −10°C, and the xerogel thus obtained was coated with platinum by vapor deposition. The accelerating voltage was 15 kV.

X-ray Powder Diffraction

The xerogel was placed into a glass capillary tube ($\varnothing = 0.7$ mm), and the X-ray diffractogram was recorded on an imaging plate using Cu radiation (1.54178 Å) at a distance of 15 cm.

FT-IR Measurement of Hydrogels

A Perkin Elmer Spectrum equipped with a universal ATR unit was used for FT-IR measurement, and all FT-IR measurements of gels were performed in the attenuated total reflection (ATR) mode. The solution, hydrogel, and xerogel from D_2O were measured on the plate of universal ATR.

References

[1] Takeuchi, M.; Ikeda, M.; Sugasaki, A.; Shinkai, S. *Acc. Chem. Res.* **2001**, *34*, 865.
[2] Pease, A. R.; Jeppesen, J. O.; Stoddart, J. F.; Luo, Y.; Collier, C. P.; Heath, A. J. R. *Acc. Chem. Res.* **2001**, *34*, 433.
[3] de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
[4] Schalley, C. A.; Beizai, K.; Vögtle, F. *Acc. Chem. Res.* **2001**, *34*, 465.
[5] Collin, J.-P.; Dietrich-Buchecker, C.; Gaviña, P.; Jimenez-Molero, M. C.; Sauvage, J.-P. *Acc. Chem. Res.* **2001**, *34*, 477.
[6] Feringa, B. L. *Acc. Chem. Res.* **2001**, *34*, 504.
[7] Eddaoudi, M.; Moler, D. B.; Li, H.; Chen, B.; Reineke, T. M.; O'Keeffe, M.; Yaghi, O. M. *Acc. Chem. Res.* **2001**, *34*, 319.
[8] Kahn, O. *Acc. Chem. Res.* **2000**, *33*, 647.
[9] Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071.
[10] Fouquey, C.; Lehn, J.-M.; Levelut, A.-M. *Adv. Mater.* **1990**, *2*, 254.
[11] Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer, B. J. B.; Ky Hirschberg, J. H. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. *Science* **1997**, *278*, 1601.
[12] Castellano, R. K.; Rudkevich, D. M.; Rebek, J. Jr, *Proc. Natl Acad. Sci. USA* **1997**, *94*, 7132.
[13] Hanabusa, K.; Yamada, M.; Kimura, M.; Shirai, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1949.
[14] Yoza, K.; Amanokura, N.; Ono, Y.; Akao, T.; Shinmori, H.; Takeuchi, M.; Shinkai, S.; Reinhoudt, D. N. *Chem. Eur. J.* **1999**, *5*, 2722.
[15] Bhattacharya, S.; Krishnan-Ghosh, Y. *Chem. Commun.* **2001**, 185.
[16] Terech, P.; Weiss, R. G. *Chem. Rev.* **1997**, *97*, 3133.
[17] Menger, F. M.; Caran, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 11679.
[18] Estroff, L. A.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447.
[19] Makarevic, J.; Jokic, M.; Peric, B.; Tomisic, V.; Kojic-Prodic, B.; Zinic, M. *Chem. Eur. J.* **2001**, *7*, 3328.
[20] Jung, J. W.; John, G.; Masuda, M.; Yoshida, K.; Shinkai, S.; Shimizu, T. *Langmuir* **2001**, *17*, 7229.
[21] Kobayashi, H.; Friggeri, A.; Koumoto, K.; Amaike, M.; Shinkai, S.; Reinhoudt, D. N. *Org. Lett.* **2002**, *4*, 1423.
[22] Hamachi, I.; Kiyonaka, S.; Shinkai, S. *Chem. Commun.* **2000**, 1281.
[23] Hamachi, I.; Kiyonnaka, S.; Shinkai, S. *Tetrahedron Lett.* **2001**, *42*, 6141.
[24] Kiyonaka, S.; Shinkai, S.; Hamachi, I. *Chem. Eur. J.* **2003**, *9*, 976.
[25] Kiyonaka, S.; Sugiyasu, K.; Shinkai, S.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 10954.

[26] Hirotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392.
[27] Suzuki, A.; Tanaka, T. *Nature* **1990**, *346*, 345.
[28] Kokufata, E.; Zhang, Y.-Q.; Tanaka, T. *Nature* **1991**, *351*, 302.
[29] Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.
[30] Pelton, R. *Adv. Colloid Interface Sci.* **2000**, *85*, 1.
[31] Jayaraman, N.; Stoddart, J. F. *Tetrahedron Lett.* **1997**, *38*, 6767.
[32] Dahmén, J.; Frejd, T.; Grönberg, G.; Lave, T.; Magnusson, G.; Noori, G. *Carbohydr. Res.* **1983**, *116*, 303.
[33] Arsequell, G.; Krippner, L.; Dwek, R. A.; Wong, S. Y. C. *J. Chem. Soc., Chem. Commun.* **1994**, 2383.
[34] Hansen, H. C.; Haataja, S.; Finne, J.; Magnusson, G. *J. Am. Chem. Soc.* **1997**, *119*, 6974.