CHEMISTRY
AN ASIAN JOURNAL
www.chemasianj.org

Accepted Article

Title: Enzymatic Hydrolysis-Responsive Supramolecular Hydrogels Composed of Maltose-Coupled Amphiphilic Ureas

Authors: Masamichi Yamanaka, Ryohei Yoshisaki, Shinya Kimura, and Masashi Yokoya

To be cited as: *Chem. Asian J.* 10.1002/asia.202100376

Link to VoR: https://doi.org/10.1002/asia.202100376

A Journal of
ACES Asian Chemical Editorial Society

A sister journal of Angewandte Chemie and Chemistry – A European Journal

WILEY-VCH

10.1002/asia.202100376

WILEY-VCH

FULL PAPER

Enzymatic Hydrolysis-Responsive Supramolecular Hydrogels Composed of Maltose-Coupled Amphiphilic Ureas

Ryohei Yoshisaki,[a,b] Shinya Kimura, Masashi Yokoya, and Masamichi Yamanaka*[a]

[a] R. Yoshisaki, Dr. S. Kimura, Dr. M. Yokoya, Prof. Dr. M. Yamanaka
Meiji Pharmaceutical University
2-522-1 Noshio, Kiyose, Tokyo 204-8588 (Japan)
E-mail: yamanaka@my-pharm.ac.jp
[b] R. Yoshisaki,
Department of Chemistry, Shizuoka University
836 Ohya, Suruga-ku, Shizuoka 422-8529 (Japan)

Supporting information for this article is given via a link at the end of the document.

Abstract: Maltose is a ubiquitous disaccharide produced by the hydrolysis of starch. Amphiphilic ureas bearing hydrophilic maltose moiety were synthesized via the following three steps: I) construction of urea derivatives by the condensation of 4-nitrophenyl isocyanate and alkylamines, II) reduction of the nitro group by hydrogenation, and III) an aminoglycosylation reaction of the amino group and the unprotected maltose. These amphiphilic ureas functioned as low molecular weight hydrogelators, and the mixtures of the amphipathic ureas and water formed supramolecular hydrogels. The gelation ability largely depended on the chain length of the alkyl group of the amphiphilic urea; amphipathic urea having a decyl group had the highest gelation ability (minimum gelation concentration = 0.4 mM). The physical properties of the supramolecular hydrogels were evaluated by measuring their thermal stability and dynamic viscoelasticity. These supramolecular hydrogels underwent gel-to-sol phase transition upon the addition of α-glucosidase as a result of the α-glucosidase-catalyzed hydrolysis of the maltose moiety of the amphipathic urea.

Introduction

Sugars, which have excellent structural diversity and high hydrophilicity, have been widely applied as a component of supramolecular architectures involving gelatinous materials.[1,2] Monosaccharides are often used in such studies because they are easy to obtain and handle. However, it is desirable to apply disaccharides, trisaccharides, and oligosaccharides for the development of materials that exhibit higher-order functions. Maltose is a disaccharide in which two molecules of glucose are joined by an α-1,4-glycosidic bond. Starch, whose linear portion is composed of repeating maltose groups, affords maltose by enzymatic hydrolysis, making maltose one of the most readily available disaccharides.[3] The application of maltose as a substrate for functional materials would be an effective use of this ubiquitous disaccharide.[4–10]

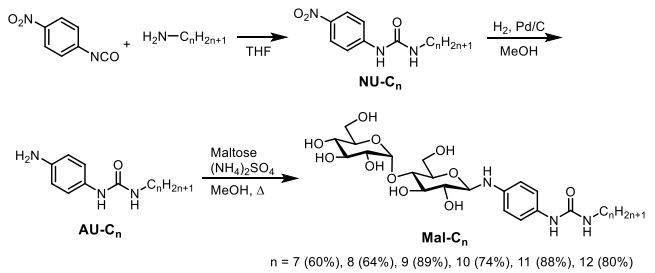
An efficient synthesis that has only a few steps is an important factor in the development of materials for commercial use. However, when synthesizing a molecule bearing a sugar moiety, it is generally necessary to protect the hydroxyl groups, which increases the number of synthetic steps. An aminoglycosylation reaction, which produces a *N*-linked glycoside by the reaction of a sugar and an amine, can be applied even to unprotected sugars making it an effective route for the efficient synthesis of the target molecule.[11–13] We have previously reported the synthesis of low molecular weight hydrogelators (LMWHGs) using an aminoglycosylation reaction with unprotected lactose.[14,15] The self-assembly of LMWHGs can form supramolecular hydrogels, which have received significant attentions in recent decades because of their broad applications, such as in drug delivery systems (DDS), regenerative medicine, and cell cultures.[1,16–19] Supramolecular hydrogels that respond to stimuli present in the body, such as temperature, pH, redox, and enzymes, are an important feature of these applications.[20,21] Herein, we report the synthesis of amphiphilic urea derivatives having maltose as a hydrophilic group that functioned as LMWHGs. The resulting supramolecular hydrogels showed gel-to-sol phase transition in response to enzymatic hydrolysis of the maltose moiety.

Results and Discussion

Synthesis of Amphiphilic Ureas

Most LMWHGs are amphiphilic molecules that achieve regulated self-assembly in aqueous media. Amphiphilic ureas (**Mal-C$_n$**) with a hydrophilic maltose moiety were designed as LMWHG candidates. **Mal-C$_n$** can be synthesized in three steps from commercially available compounds via an aminoglycosylation reaction with unprotected maltose (Scheme 1). The hydrophobic urea structure (**NU-C$_n$**) was obtained via the condensation of 4-nitrophenyl isocyanate and alkylamine.[15] Reactions were carried out using amines with alkyl chain lengths from heptyl (**C$_7$**) to dodecyl (**C$_{12}$**). The nitro group of **NU-C$_n$** was reduced to an amino group by hydrogenation in the presence of a palladium/carbon catalyst to obtain the desired amine (**AU-C$_n$**).[15] The aminoglycosylation reaction of the amino group of **AU-C$_n$** with maltose proceeded in the presence of a catalytic amount of ammonium sulfate to give the desired amphiphilic urea (**Mal-C$_n$**). The reactions occurred in a stereoselective manner, and only β-isomers were obtained as products.[12] The structures of **Mal-C$_n$** were confirmed via ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

10.1002/asia.202100376



Scheme 1. Synthesis of Amphiphilic Ureas Mal-C_n.

Gelation Experiments

The gelation abilities of the amphiphilic ureas **Mal-C_n** are shown in Table 1. A mixture of **Mal-C_n** and water in a glass vial was heated until the **Mal-C_n** dissolved, whereupon the mixture was cooled slowly at room temperature. When the glass vial was inverted, the mixture remaining in the vial was defined as the gel. The amphiphilic urea with a heptyl group (**Mal-C_7**) did not form a supramolecular hydrogel, even at a high concentration of 40 mM. The amphipathic urea having an octyl group (**Mal-C_8**) functioned as a LMWHG and formed a supramolecular hydrogel at a minimum gelation concentration (MGC) of 1.5 mM. Amphipathic ureas having nonyl and decyl groups (**Mal-C_9** and **Mal-C_{10}**) had better gelation ability than **Mal-C_8**, and amphipathic ureas with long alkyl groups (**Mal-C_{11}** and **Mal-C_{12}**) had worse gelation ability than **Mal-C_8** (Table 1).

The gelation ability of the amphipathic ureas was greatly influenced by the chain length of the alkyl group, **Mal-C_{10}** showed the highest gelation ability (MGC = 0.4 mM); the MGCs of **Mal-C_n** ureas having both shorter (n = 8 and 9) and longer (n = 11 and 12) alkyl groups were higher than that of **Mal-C_{10}**. The balance between hydrophilicity and hydrophobicity of a LMWHG is extremely important in the formation of supramolecular hydrogels, and a difference of one carbon chain had a dramatic effect on gelation ability.

Table 1. Minimum Gelation Concentrations (MGCs) and Phase Transition Temperatures (T_{gel}) of **Mal-C_n**.

	MGC (mM)	T_{gel} (ºC) [a]
Mal-C_7	-	-
Mal-C_8	1.5	52–58
Mal-C_9	1.0	54–58
Mal-C_{10}	0.4	62–70
Mal-C_{11}	2.3	46–56
Mal-C_{12}	5.3	34–52

[a] The phase transition temperatures of 10 mM supramolecular hydrogels of **Mal-C_n** were measured.

Properties of Supramolecular Hydrogels

The thermal stability of the supramolecular hydrogels was evaluated by measuring gel-to-sol phase transition temperature (T_{gel}) (Tables 1 and S1). T_{gel} was determined by the inverse flow method and defined as the temperature at which the gel fell down the vial.[22] The temperature of the oil bath was increased slowly at a rate of 0.2 °C/min. The T_{gel} of a 2.0 mM supramolecular hydrogel of **Mal-C_8** had a wide temperature range of 28–44 °C. Under constant temperature conditions of 35 and 40 °C, a 2.0 mM supramolecular hydrogel of **Mal-C_8** required 80 and 60 min, respectively, to complete the gel-to-sol phase transition. When the concentration of **Mal-C_8** was increased to 5.0 and 10 mM, the T_{gel} increased to 44–56 and 52–58 °C, respectively, and the temperature ranges became narrower. Similar trends were observed for the supramolecular hydrogels of **Mal-C_9** and **Mal-C_{10}**. The T_{gel} values of the 2.0, 5.0, and 10 mM supramolecular hydrogels of **Mal-C_9** were 28–42, 38–56, and 54–58 °C, respectively. The T_{gel} values of the 2.0, 5.0, and 10 mM supramolecular hydrogels of **Mal-C_{10}** were 26–40, 34–52, and 62–70 °C, respectively. Supramolecular hydrogels of **Mal-C_{11}** and **Mal-C_{12}**, which have long alkyl chains, tended to have lower T_{gel} values. The 5 and 10 mM supramolecular hydrogels of **Mal-C_{11}** showed T_{gel} values of 26–40, and 46–56 °C, respectively. The T_{gel} of 10 mM supramolecular hydrogels of **Mal-C_{12}** was 34–52 °C. A good correlation was found between gelation ability and T_{gel} when 10 mM supramolecular hydrogels of **Mal-C_n** were compared (Table 1).

The physical properties of the supramolecular hydrogels of **Mal-C_n** were investigated by measuring their viscoelastic features using a rheometer.[23,24] To obtain receiving reproducible results, 10 mM supramolecular hydrogels were used for **Mal-C_8**, **Mal-C_9**, and **Mal-C_{10}**, and 20 mM supramolecular hydrogels were used for **Mal-C_{11}** and **Mal-C_{12}**. Both the storage moduli (G') and loss moduli (G'') were almost independent of the frequency from 0.01 to 10.0 Hz in supramolecular hydrogels of **Mal-C_8**, **Mal-C_9**, and **Mal-C_{10}**, which is typical of a supramolecular gel (Figure 1a).[25] The G' and G'' values of the 10 mM **Mal-C_8** gel are 2490 and 257 Pa, respectively, at 1.0 Hz. The G' and G'' values of the 10 mM **Mal-C_9** gel are 2950 and 380 Pa, respectively, at 1.0 Hz. The G' and G'' values of the 10 mM **Mal-C_{10}** gel are 2180 and 444 Pa, respectively, at 1.0 Hz. Comparing these three supramolecular hydrogels, there was a tendency for the tanΔ (G''/G') to increase as the alkyl chain length increased. For the supramolecular hydrogels of **Mal-C_{11}** and **Mal-C_{12}**, the G' values were frequency-dependent and increased as the frequency increased (Figure 1b). This is a typical trend observed for weak supramolecular gels.[26] Even in a 20 mM supramolecular hydrogel of **Mal-C_{11}**, the G' values were small, and the tanΔ values were large (tanΔ: 0.32 at 0.1 Hz, 0.22 at 1.0 Hz). **Mal-C_{12}** had properties similar to those of **Mal-C_{11}**. The strain sweep of the supramolecular hydrogels of **Mal-C_n** demonstrated an elastic response that is typical of supramolecular hydrogels (Figures S1 and S2).

10.1002/asia.202100376

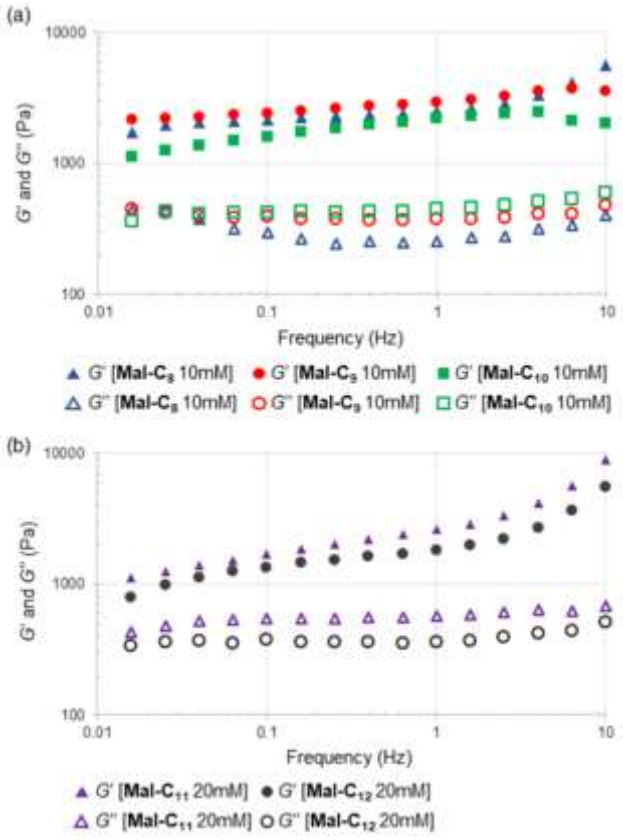


Figure 1. Storage moduli (*G′*) and loss moduli (*G″*) of supramolecular hydrogels via frequency sweep at a strain of 0.1%: (a) 10 mM of **Mal-C$_8$**, **Mal-C$_9$**, and **Mal-C$_{10}$**; (b) 20 mM of **Mal-C$_{11}$** and **Mal-C$_{12}$**.

Enzymatic Responsive Phase Transition

A gel-to-sol phase transition in response to external stimuli is a characteristic property of supramolecular gels formed by highly reversible intermolecular interactions.[20,21,27–30] The responsive stimulus can be designed by choosing the functional group to be introduced to the molecule. The development of supramolecular hydrogels that respond to stimuli existing in the living body is an important factor in various applications such as DDS. We expected that **Mal-C$_n$** would show a gel-to-sol phase transition in response to enzymatic hydrolysis of the maltose moiety.

α-Glucosidase is an enzyme that catalyzes the hydrolysis of the α-1,4-glucoside bond of sugars.[31] α-Glucosidase is present in many organisms; in humans, it is primarily expressed in the small intestine. When α-glucosidase acts on **Mal-C$_n$**, the glucosidic bond at the maltose moiety is hydrolyzed to produce amphipathic urea (**Glu-C$_n$**) and glucose (Figure 2a). Previous studies have shown that **Glu-C$_8$** does not function as a LMWHG,[15] so we used the supramolecular hydrogel of **Mal-C$_8$** to evaluate the enzymatic responsiveness. When 10 units of α-glucosidase were added to a 5 mM supramolecular hydrogel of **Mal-C$_8$** at room temperature, the supramolecular hydrogel at the interface with α-glucosidase collapsed. After 8 h, the mixture completely changed from a hydrogel to a suspension containing a precipitate (Figure 2b). The amount of enzyme greatly affected the time required for the phase transition; using 1 unit of α-glucosidase took 5 days for the complete phase transition of a 5 mM supramolecular hydrogel of **Mal-C$_8$**. The process of supramolecular hydrogel transformation into a suspension could also be followed by dynamic viscoelasticity measurements using a rheometer (Figure 2c). After the addition of α-glucosidase to the supramolecular hydrogel, *G′* decreased over time, and *G″* increased over time. After approximately 3 h, the value of *G″* exceeded that of *G′*. The difference in the time required for the phase transition between that determined by examination of the glass vial and that determined by rheometer measurements is probably due to the thickness of the sample. Structural changes in **Mal-C$_8$** as a result of α-glucosidase were monitored by ^{1}H NMR (Figure 2d). The ^{1}H NMR spectra showed that the amphiphilic urea **Glu-C$_8$**, which formed from hydrolysis of the **Mal-C$_8$** maltose moiety, was a major component of the suspension obtained after mixing a supramolecular hydrogel of **Mal-C$_8$** and α-glucosidase. The suspension also contained ~35% **AU-C$_8$**, which resulted from hydrolysis of the *N*-glycosidic bond of **Mal-C$_8$**.

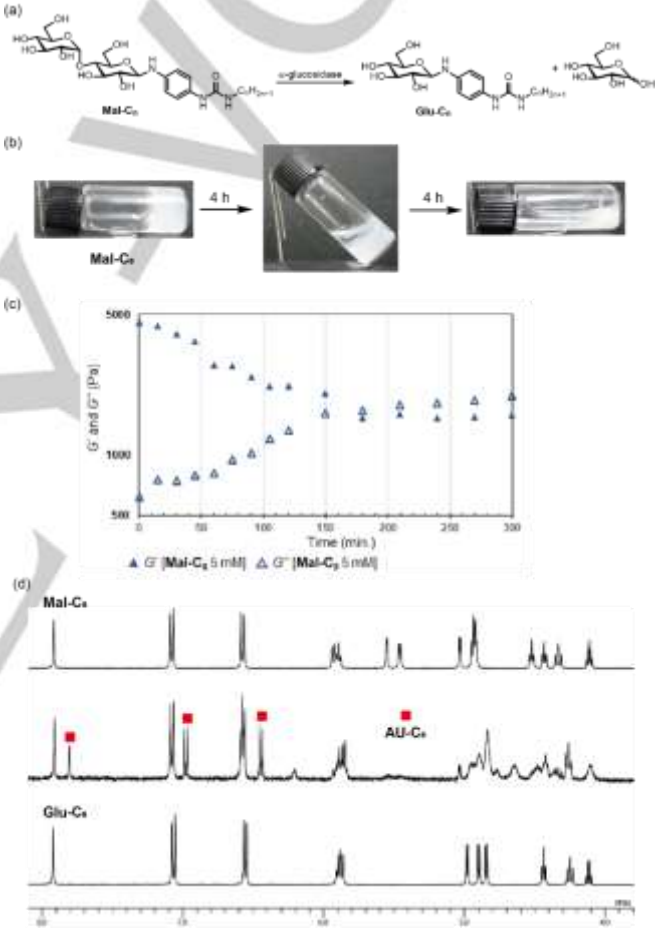


Figure 2. (a) Reaction scheme of the enzymatic hydrolysis of **Mal-C$_n$**, (b) Photographs showing the time course of a mixture of supramolecular hydrogel (5.0 mM **Mal-C$_8$**) and α-glucosidase (10 units) after 0, 4, and 8 h, (c) Storage moduli (*G′*) and loss moduli (*G″*) of a mixture of **Mal-C$_8$** and α-glucosidase (6 units) via time sweep at a strain of 0.1% and a frequency of 1.0 Hz, (d) ^{1}H NMR spectra (DMSO-*d*$_6$, 298 K) of **Mal-C$_8$** (top), a mixture of a supramolecular hydrogel of **Mal-C$_8$** and α-glucosidase after 8 h (middle), and **Glu-C$_8$** (bottom).

Supramolecular hydrogels of **Mal-C$_9$** and **Mal-C$_{10}$** showed similar gel-to-sol phase transitions in response to enzymatic hydrolysis. A 1 mM supramolecular hydrogel of **Mal-C$_9$** became a suspension after 3 h at room temperature in the presence of 10 units of α-glucosidase (Figure 3a). A 0.5 mM supramolecular hydrogel of **Mal-C$_{10}$** also underwent phase transition to a

suspension after 3 h at room temperature in the presence of 10 units of α-glucosidase (Figure S3). The rate of the phase transition could be controlled by not only the amount of enzyme but also an external additive. Acarbose is an inhibitor of α-glucosidase and therefore inhibits the hydrolysis of saccharides.[32,33] It was believed that if acarbose was allowed to coexist in a supramolecular hydrogel, the hydrolysis of **Mal-C$_n$** catalyzed by α-glucosidase would be delayed, and the phase transition would require more time. Indeed, the addition of 10 units of α-glucosidase to a 1 mM supramolecular hydrogel of **Mal-C$_9$** containing 1 mM acarbose resulted in the phase transition taking 18 h to complete (Figure 3b). When 10 units of α-glucosidase were added to a 1 mM supramolecular hydrogel of **Mal-C$_{10}$** containing 1 mM acarbose, the phase transition also took 18 h to complete (Figure S4).

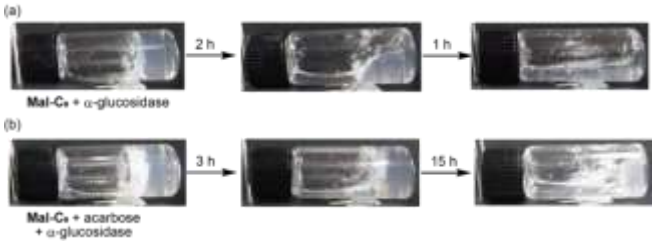


Figure 3. Photographs showing the time course of a mixture of (a) a supramolecular hydrogel (1.0 mM of **Mal-C$_9$**) and α-glucosidase (10 units) after 0, 2, and 3 h; (b) a supramolecular hydrogel mixed with an enzyme inhibitor (1.0 mM of **Mal-C$_9$** and 1.0 mM of acarbose) and α-glucosidase (10 units) after 0, 3, and 18 h.

Conclusion

Amphipathic ureas were synthesized in only three steps from commercially available compounds by applying an aminoglycosylation reaction using an unprotected sugar (maltose). The synthesized amphipathic ureas functioned as LMWHGs. Their gelation abilities largely depended on their alkyl chain length; the amphipathic urea having a decyl group showed the highest gelation ability (MGC = 0.4 mM). The supramolecular hydrogels collapsed after the addition of α-glucosidase, and the phase transitions were complete after several hours. ^{1}H NMR analysis revealed that the phase transition proceeded mainly by the α-glucosidase-catalyzed hydrolysis of the maltose moiety of the amphipathic urea. The rate of the phase transitions could be controlled by not only the amount of enzyme but also the addition of an enzyme inhibitor (acarbose). Since α-glucosidase is an enzyme expressed in the small intestine of humans, the supramolecular hydrogel is expected to apply as a carrier of drugs to the small intestine. Further research on maltose-based amphiphilic ureas is underway in our laboratory.

Experimental Section

Synthesis of Mal-C$_n$

Urea derivatives **AU-C$_n$** were synthesized in two steps from 4-nitrophenyl isocyanate and alkylamines according to our previously reported procedure. [15] A mixture of **AU-C$_n$**, maltose monohydrate, and $(NH_4)_2SO_4$ in MeOH was stirred at 50 ºC for 43 h under an argon atmosphere. The reaction mixture was cooled to room temperature and ethyl acetate was added. The obtained solid was collected by filtration and washed with MeOH that had been cooled to 0 ºC. The desired **Mal-C$_n$** was obtained as a white solid.

Gelation experiment

A mixture of **Mal-C$_n$** and H_2O in a glass vial was heated on a hot plate (150 °C) until the **Mal-C$_n$** dissolved. The resulting solution was gradually cooled to ambient temperature. Gel formation was evaluated by the inverted tube test. When the vial was inverted, any mixture remaining in the vial was defined as gel.

Acknowledgements

This work was supported by Grant-in-aid for the Scientific Research (No. 20K06977 for M.Yo.; 17H06374 and 21K06485 for M.Ya.) the Japan Society for the Promotion of Science (JSPS) or the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the NOVARTIS Foundation (Japan) for the Promotion of Science (for S.K.).

Keywords: gels • hydrogels • phase transition • self-assembly • urea

[1] S. Datta, S. Bhattacharya, *Chem. Soc. Rev.* **2015**, *44*, 5596–5637.
[2] M. Delbianco, P. Bharate, S. Varela-Aramburu, P. H. Seeberger, *Chem. Rev.* **2016**, *116*, 1693–1752.
[3] A. M. Smith, S. C. Zeeman, S. M. Smith, *Annu. Rev. Plant Biol.* **2005**, *56*, 73–98.
[4] A. Panacek, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V. K. Sharma, T. Nevecna, R. Zboril, *J. Phys. Chem. B* **2006**, *110*, 16248–16253.
[5] Z. Guo, A. Lei, Y. Zhang, Q. Xu, X. Xue, F. Zhang, X. Liang, *Chem. Commun.* **2007**, 2491–2493.
[6] C. S. Kolli, A. K. Banga, *Pharm. Res.* **2008**, *25*, 104–113.
[7] M. Fuss, M. Luna, D. Alcantara, J. M. Fuente, S. Penades, F. Briones, *Langmuir* **2008**, *24*, 5124–5128.
[8] M. J. Clemente, J. Fitremann, M. Mauzac, J. L. Serrano, L. Oriol, *Langmuir* **2011**, *27*, 15236–15247.
[9] X. Ma, L. Li, L. Yang, C. Su, K. Wang, S. Yuan, J. Zhou, *J. Hazard. Mater.* **2012**, *209-210*, 467–477.
[10] W. Ma, L. Xu, Z. Li, Y. Sun, Y. Bai, H. Liu, *Nanoscale* **2016**, *8*, 10908–10912.
[11] S. Bhattacharya, S. N. G. Acharya, *Chem. Mater.* **1999**, *11*, 3504–3511.
[12] N. Bridiau, M. Benmansour, M. D. Legoy, T. Maugard, *Tetrahedron* **2007**, *63*, 4178–4183.
[13] L. Zhang, Y. Dong, X. Zhang, X. Guo, *J. Chem. Eng. Data* **2016**, *61*, 2969–2978.
[14] S. Akama, T. Maki, M. Yamanaka, *Chem. Commun.* **2018**, *54*, 8814–8817.
[15] T. Maki, R. Yoshisaki, S. Akama, M. Yamanaka, *Polym. J.* **2020**, *52*, 931–938.
[16] L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201–1218.
[17] M. de Loos, B. L. Feringa, J. H. van Esch, *Eur. J. Org. Chem.* **2005**, 3615–3631.
[18] A. R. Hirst, B. Escuder, J. F. Miravet, D. K. Smith, *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 8002–8018.
[19] X. Du, J. Zhou, J. Shi, B. Xu, *Chem. Rev.* **2015**, *115*, 13165–13307.
[20] M. Ikeda, *Polym. J.* **2019**, *51*, 371–380.
[21] S. Kimura, M. Yokoya, M. Yamanaka, *Chem. Lett.* **2021**, *50*, 459–466.
[22] M. George, R. G. Weiss, *Langmuir* **2002**, *18*, 7124–7135.
[23] A. Dawn, H. Kumari, *Chem Eur. J.* **2018**, *24*, 762–776.
[24] J. Takeshita, Y. Hasegawa, K. Yanai, A. Yamamoto, A. Ishii, M. Hasegawa, M. Yamanaka, *Chem. Asian J.* **2017**, *12*, 2029–2032.

10.1002/asia.202100376

[25] X. Li, J. Li, Y. Gao, Y. Kuang, J. Shi, B. Xu, *J. Am. Chem. Soc.* **2010**, *132*, 17707–17709.
[26] A. J. Wright, A. G. Marangoni, *J. Am. Oil Chem. Soc.* **2006**, *83*, 497–503.
[27] F. Zhao, C. S. Weitzel, Y. Gao, H. M. Browdy, J. Shi, H.-C. Lin, S. T. Lovett, B. Xu, *Nanoscale* **2011**, *3*, 2859–2861.
[28] R. Ochi, K. Kurotani, M. Ikeda, S. Kiyonaka, I. Hamachi, *Chem. Commun.* **2013**, *49*, 2115–2117.
[29] H. Shigemitsu, I. Hamachi, *Acc. Chem. Res.* **2017**, *50*, 740–750.
[30] N. Falcone, H. B. Kraatz, *Chem Eur. J.* **2018**, *24*, 14316–14328.
[31] S. Chiba, *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1233–1239.
[32] A. E. Martin, P. A. Montgomery, *Am. J. Health Syst. Pharm.* **1996**, *53*, 2277–2290.
[33] U. F. Wehmeier, W. Piepersberg, *Appl. Microbiol. Biotechnol.* **2004**, *63*, 613–625.

10.1002/asia.202100376

Entry for the Table of Contents

Amphiphilic ureas bearing hydrophilic maltose moiety functioned as low molecular weight hydrogelators. The gelation ability largely depended on the chain length of the alkyl group of the amphiphilic urea; amphipathic urea having a decyl group had the highest gelation ability (minimum gelation concentration = 0.4 mM). These supramolecular hydrogels underwent gel-to-sol phase transition upon the addition of α-glucosidase as a result of the hydrolysis of the maltose moiety.