**In Vitro Synthesis of Branchless Linear (1 → 6)-α-D-Glucan by Glucosyltransferase K: Mechanical and Swelling Properties of Its Hydrogels Crosslinked with Diglycidyl Ethers**

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**ABSTRACT:** A hydrogel was prepared from a polysaccharide, enzymatically synthesized through a one-pot reaction in aqueous solution, and its properties as a functional material were evaluated. Enzymatic synthesis using glucosyltransferase K obtained from *Streptococcus salivarius* ATCC 25975 was performed with sucrose as a substrate. The synthetic product was unbranched linear (1 → 6)-α-D-glucan with a high molecular weight, $M_w$: 1.0–3.0 $\times$ 10$^5$. The synthesized (1 → 6)-α-D-glucan was insoluble in water and crystallized in a monoclinic unit cell, which is consistent with the hydrated form of dextran. Transparent and highly swellable (1 → 6)-α-D-glucan hydrogels were obtained by crosslinking with diglycidyl ethers. The hydrogels showed no syneresis and no volume change during compression, resulting in the retention of shape under repeated compression. The elastic moduli of these hydrogels (<60 kPa) are smaller than those of other polysaccharide-based hydrogels having the same solid contents. The oven-dried gels could be restored to the hydrogel state with the original transparency and a recovery ratio greater than 98%. The mechanism of water diffusion into the hydrogel was investigated using the kinetic equation of Peppas. The properties of the hydrogel are impressive relative to those of other polysaccharide-based hydrogels, suggesting its potential as a functional biomaterial.

**INTRODUCTION**

Dextran is a water-soluble polysaccharide consisting of linear α-(1 → 6)-D-linked glucose as a backbone with branches extending mainly from α-(1 → 3) and occasionally from α-(1 → 4) or α-(1 → 2) linkages. Several Gram-positive bacteria and facultatively anaerobic cocci, such as *Leuconostoc* and *Streptococcus*, produce dextran as an exopolysaccharide from sucrose. The degree of branching and molecular weight depend on the bacterial strain and culture conditions. However, natural dextran produced from bacteria always has a degree of branching of at least 5%. Dextran is biocompatible and biodegradable and can be degraded by dextran-1,6-glucosidase and dextranase in the human body. Therefore, it is used in various fields, especially the medical and pharmaceutical fields.

In vitro synthesis by genetic engineering enzymes is another way to produce dextran. Glucosyltransferase (Gtf), also known as glucansucrase, can catalyze glucan synthesis from sucrose and is an enzyme in the glycoside hydrolase family 70 (GH70). Several Gtf enzymes that synthesize dextran have been obtained from lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Weissella*, and *Streptococcus*. The chemical structure of the synthesized dextran varies depending on the enzyme, varying from 5 to 50% branching from α-(1 → 3), α-(1 → 4), or α-(1 → 2) linkages. There are also several reports of Gtf's that synthesize pure (1 → 6)-α-D-glucan as a hydrogel.
However, only characterization of these enzymes has been performed, and the study of the synthesized (1 → 6)-α-D-glucan as a material has not been evaluated. In particular, functional materials prepared from linear (1 → 6)-α-D-glucan have the potential to achieve different properties to those of the native branched dextran.

Hydrogels prepared by the three-dimensional crosslinking of polysaccharides are used in a variety of applications because of their unique properties such as softness and flexibility, high water absorption, and high substrate adsorption. Although there are various crosslinking agents, ethylene glycol diglycidyl ether (EGDE) is widely used not only for polysaccharides but also for crosslinking biopolymers such as DNA and proteins. This is because EGDE has low toxicity and undergoes ring-opening reactions with various functional groups such as hydroxyl, carboxyl, amino, and sulfhydryl groups under alkaline conditions. Because the reaction proceeds in aqueous solution, EGDE is an environmentally friendly crosslinking agent for polysaccharide hydrogels.

In this study, the recombinant glucosyltransferase K (GtfK) enzyme was prepared from Streptococcus salivarius ATCC 25975, a bacterium typically present in the oral cavity that promotes dental plaque formation and carries induction. Linear (1 → 6)-α-D-glucan was synthesized in vitro using recombinant GtfK from sucrose in an aqueous solution (Scheme 1). The effect of enzymatic synthetic conditions on the molecular weight and yield of synthesized (1 → 6)-α-D-glucan, as well as the solid-state structure of the glucan, was investigated. In addition, (1 → 6)-α-D-glucan was crosslinked with EGDE and poly(ethylene glycol) diglycidyl ethers (P200 and P400, vide infra) to obtain transparent hydrogels (Scheme 1). After analyzing the structure of the hydrogels, the mechanical and swelling properties were investigated.

**RESULTS AND DISCUSSION**

**Chemical Structure of Synthesized (1 → 6)-α-D-Glucan.** (1 → 6)-α-D-Glucan was synthesized from sucrose (400 mM) using recombinant GtfK (0.05 U/mL) in 50 mM phosphate buffer (pH 6.0) at 37 °C for 3 days. During the synthesis of (1 → 6)-α-D-glucan, the medium was transparent and gradually became more viscous as the reaction time increased. However, the (1 → 6)-α-D-glucan precipitated in ethanol was insoluble in water even when heated.

One-dimensional 1H and 13C NMR and two-dimensional 1H−1H correlation spectroscopy (COSY), 1H−13C heteronuclear single−quantum correlation spectroscopy (HSQC), and 1H−13C heteronuclear multiple−bond correlation (HMBC) NMR measurements were conducted to determine the primary chemical structure of the synthesized glucan (Figures 1 and S2). The chemical shifts in the 1H and 13C NMR spectra were assigned with the help of the two-dimensional NMR spectra and were in good agreement with those of (1 → 6)-α-D-glucan reported by Simpson et al. (1995). The broad peaks at around 4.47 and 4.92 ppm in the 1H NMR spectrum can be attributed to hydroxyl protons on C2 and C3 and C4 (for which the latter peaks overlap), respectively. In the 13C NMR spectrum, the peaks corresponding to C4 and C5 overlapped at 70.32 ppm, and all peaks were singlets. These results reveal that the synthesized glucan had a pure linear structure linked with uniform glycosidic bonds without branching. Compared with water-soluble dextran with a degree of branching of about 5%, the water-insolubility of (1 → 6)-α-D-glucan synthesized by GtfK is possibly due to its branchless linear structure.
Effect of Synthetic Condition on the Molecular Weight and Yield of (1 → 6)-α-D-Glucan. To investigate the effect of the synthetic conditions on the molecular weight and yield of (1 → 6)-α-D-glucan, enzymatic reactions for 3 days were performed by changing only a single factor: sucrose concentration (100–800 mM), enzyme concentration (0.01−0.2 U/mL), or reaction temperature (20−40 °C) (Figure 2). When the sucrose concentration was changed, the molecular weight and dispersity decreased with increasing sucrose concentrations, but the yield was maximized at 400 mM (80%). When the enzyme concentration was 0.01 U/mL, the molecular weight and dispersity were high (M_w = 3.0 × 10^5, D_M ≈ 2.7), but the yield was extremely low, about 1%. However, at other enzyme concentrations, the molecular weight, dispersity, and yield were almost constant: M_w ≈ 1.5 × 10^5, D_M ≈ 2.3, and 80%, respectively. When the reaction temperature was changed, the molecular weights were slightly higher at 25 °C and 30 °C (M_w = 2.0 × 10^5) and almost constant at other temperatures (M_w = 1.5 × 10^5). The dispersity was around D_M = 2.3, and the yield increased from 2% at 20 °C to 80% at 37 °C with increasing reaction temperature. These results indicate that the molecular weight can be controlled by changing the enzyme reaction conditions, M_w = 1.0−3.0 × 10^5. Thus, GtfK can synthesize (1 → 6)-α-D-glucan with a M_w = 1.5 × 10^5 in a high yield of 80% under the followed reaction conditions: [sucrose] = 400 mM, [enzyme] = 0.05 U/mL, and T = 37 °C. Notably, the yield of 80% is much higher than 12% of (1 → 3)-α-D-glucan synthesized by GtfJ, another enzyme of the same family.26 In the following experiment, (1 → 6)-α-D-glucan was synthesized under these conditions.

Solid-State Structure of (1 → 6)-α-D-Glucan. Although dextran is a water-soluble polysaccharide, the synthesized (1 → 6)-α-D-glucan was insoluble in water, as described above, probably because of the branchless structure. The solid structure was investigated by X-ray diffraction (XRD) and cross polarization/magic angle spinning (CP/MAS) 13C NMR spectroscopy.

To obtain highly crystalline samples for XRD, acid hydrolysis of (1 → 6)-α-D-glucan was carried out. The XRD profiles of the wet and dry samples (Figure 3a) were almost the same, indicating that both samples had similar crystalline forms. However, the XRD profile of the wet sample exhibited sharper diffraction peaks and higher crystallinity. Peak separation of the XRD profile of the wet sample was carried out to calculate the d-spacings. After indexing (Table S1), the unit cell was determined to be monoclinic having parameters a = 25.85 Å, b = 10.22 Å, c = 7.82 Å, and β = 92.02°. This unit cell is consistent with that of the hydrated form of dextran containing six chains and eight water molecules.27 The solid-state CP/MAS 13C NMR spectra of the wet and dry samples (Figure 3b) showed resonance peaks in the same positions. However, the peaks in the wet sample were sharp and resolved. These results indicate that the conformation of the molecular chain was the same in the wet and dry samples, but the wet sample had higher crystallinity and less conformational disorder. Combined with the XRD results, the CP/MAS 13C NMR spectra indicate that (1 → 6)-α-D-glucan is a crystalline hydrate when the humidity is RH 85%, and the structure was disturbed by oven-drying at 105 °C but dehydration was not complete. The C1 and C6 peaks appeared as triplets in the 92−100 and 63−67 ppm regions, respectively.

Figure 2. Effect of enzymatic conditions on the molecular weight (M_w), dispersity (D_M = M_w/M_n), and yield of (1 → 6)-α-D-glucan synthesized.

Figure 3. (a) XRD profiles and (b) solid-state CP/MAS 13C NMR spectra of wet and dry (1 → 6)-α-D-glucan.
Downfield of the C1 triplet, a small peak at 98 ppm increased in intensity after drying. Thus, the C1 peaks in triplet can be attributed to the crystalline component, and the small peak in the downfield region may be derived from the crystalline surface or the amorphous component. The triplets corresponding to C1 and C6 indicate the presence of more than three glucose residues in the asymmetric unit, which is consistent with the XRD results, indicating a six-chain monoclinic unit cell.

**Structure of (1 → 6)-α-D-Glucan Hydrogels.** Self-standing hydrogels of (1 → 6)-α-D-glucan with colorless and clear appearances (Figure 4a) were prepared using various amounts of diglycidyl ethers as crosslinkers. The swelling ratios of the hydrogels are shown in Figure 4b.

The swelling ratios of G-EGDE and G-P200 gradually decreased from 4700 to 1300 and 5000 to 3000%, respectively, with increasing crosslinker dosage. At the same crosslinker dosage, the swelling ratio increased with an increase in the molecular chain length of the crosslinker, reaching up to 8700% of G-P400-1. Although it was not possible to quantitatively evaluate the degree of crosslinking due to the self-assembling behavior of diglycidyl ethers, the reduction in the swelling ratio and the increase in the solid content with increasing crosslinker dosage clearly indicate an increase in the degree of crosslinking. Furthermore, the increase in the molecular chain length of the crosslinker led to a loose hydrogel structure that could absorb more water.

The UV–vis spectra of the hydrogels with a thickness of 3 mm are shown in Figure 4c. No obvious absorbance peak was detected in the visible wavelength region (approximately 400–750 nm), confirming that the hydrogels were colorless. Hydrogels prepared with crosslinkers having shorter chains, as well as those prepared with larger amounts of crosslinkers, showed higher transmittance. The transmittance of G-EGDE-3, G-EGDE-5, and G-P200-3 was greater than 90% in the visible light region, indicating that they are transparent. The transparency of the hydrogel can be explained by its uniform structure and high degree of swelling with water.

Figure 5a shows Fourier transform infrared (FT-IR) spectra of (1 → 6)-α-D-glucan and its representative hydrogels crosslinked with diglycidyl ethers. Typical absorption bands
for (1 → 6)-α-D-glucan include the OH stretching at 3373 cm\(^{-1}\), CH stretching at 2930 cm\(^{-1}\), and C-O-C stretching at 1153 cm\(^{-1}\), and other bands in the fingerprint region are consistent with those of dextran reported by Purama et al. (2009). In the spectra of the (1 → 6)-α-D-glucan hydrogels, the CH\(_2\) stretching band at 2876 cm\(^{-1}\) and C-O-C stretching band at 1111 cm\(^{-1}\) increased in strength with increasing dosage of crosslinkers. These results indicate that more crosslinks were formed on the addition of a large amount of crosslinkers.

**Figure 5b** shows solid-state CP/MAS \(^{13}\)C NMR spectra of representative (1 → 6)-α-D-glucan hydrogels recorded under wet conditions. The spectra of the hydrogels were composed of broad peaks, indicating that the crystal structure of the original (1 → 6)-α-D-glucan was destroyed, resulting in an amorphous structure. The peak at 98.1 ppm and the shoulder peak at 65.9 ppm can be assigned to C1 and C6 of the (1 → 6)-α-D-glucan backbone, respectively. The intensity of the small peak at 80.7 ppm increased with increasing crosslinker dosage. Although this peak could not be assigned, it could be derived from one of the carbon atoms of the (1 → 6)-α-D-glucan units bound to the crosslinkers. The peaks of the other carbon atoms of the glucan backbone and crosslinkers overlapped as a broad large peak centered at approximately 70.9 ppm. Peaks corresponding to the oxirane ring carbons at around 52 and 45 ppm were not detected. This means that the epoxy ring-opening reaction went to completion, regardless of the amount of the crosslinker added. When the crosslinker dosage was large, a multi-step epoxy ring-opening reaction occurred, and the ring-opening reaction proceeded further from the hydroxy group that had been generated by the ring-opening reaction. This is probably due to relatively high concentrations of NaOH solution (2%, w/v) used for the crosslinking reaction.

**Mechanical Properties of (1 → 6)-α-D-Glucan Hydrogels.** Compression tests were also performed on the hydrogels, and the stress-strain curves are shown in Figure 5a; the elastic moduli, fracture stresses, fracture strains, and Poisson’s ratios are summarized in Figure 6a. The elastic moduli of G-EGDE and G-P200 increased from 2.26 to 50.47 and 1.45 to 6.63 kPa, respectively, with increasing crosslinker dosage. Comparing hydrogels having the same n(crosslinker)/n(GU) ratio of 1, we found that longer molecular chains in the crosslinking agent resulted in smaller elastic moduli. The elastic modulus of G-P400-1 was lowest at 0.92 kPa. However, there was no clear trend in fracture stress with respect to the crosslinker dosage, and the values were in the range of 30–55 kPa. However, the fracture strain tended to show the opposite trend to that of the elastic modulus and became smaller when the crosslinker dosage increased.
dosage was higher. The fracture strain of G-EGDE and G-P200 decreased from 84.7 to 46.0% and from 87.4 to 63.1%, respectively, but those of G-EGDE-1, G-P200-1, and G-P400-1 were nearly the same. Interestingly, the Poisson’s ratios of the hydrogel samples were all about 0.5. This result indicated that the hydrogels were incompressible materials that did not change in volume during the applied deformation.

Figure 6b shows photographs of a hydrogel sample during compression and relaxation. Because the hydrogel (G-EGDE-2) broke at 73.2% strain (Figure 6a), the compression tests were carried out until the maximum strain at 60%. In addition, the hydrogel showed no syneresis; that is, the water inside the hydrogel was not lost during compression. When the strain was gradually removed, the hydrogel retook its original shape. This is because the hydrogel did not undergo volume changes during compression: Poisson’s ratio = 0.5. The compression tests of the hydrogel in the region below the fracture strain were repeated five times. The stress–strain curves (Figure 6b) are identical, indicating that the hydrogel maintained its shape on repeated compression–relaxation cycles. The shape recovery was observed for all other hydrogels with different crosslinkers and dosages (data not shown).

To compare the mechanical properties of the (1 → 6)-α-D-glucan hydrogels with other polysaccharide-based hydrogels, the elastic moduli were plotted against the solid content of the hydrogels, as shown in Figure 6c. Clearly, the elastic moduli of the hydrogels increased with increase in the solid content. The elastic moduli of (1 → 6)-α-D-glucan hydrogels, which are less than 60 kPa, are smaller than those of the other hydrogels having the same solid content. This is probably due to the flexible structure of the (1 → 6)-α-D-glucan and diglycidyl ethers crosslinking agents, which have low barriers for rotation around the glucosidic linkages and the –C–C–O– bonds, respectively.

Swelling Properties of (1 → 6)-α-D-Glucan Hydrogels.
After drying an (1 → 6)-α-D-glucan hydrogel (G-EGDE-2) in the oven, the sample shrank considerably and became opaque (Figure 7a). The dried sample was swollen again by immersion in deionized water. All oven-dried gels recovered their original transparencies, shapes, and sizes within 3 days. The recovery ratios of the representative hydrogels, R (%), are listed in Table 1. The values are all above 98%, indicating that the oven-dried samples almost recovered their initial states after swelling in water.

To investigate the mechanism of water diffusion in the (1 → 6)-α-D-glucan hydrogels, the water swelling kinetics of oven-dried samples were studied. The dynamic water swelling data for the representative hydrogels are shown in Figure 7b. The data were well fitted to a simple power law equation: Peppas kinetic model. The diffusion exponent n and diffusion constant k calculated by the least squares method are listed in Table 1. The n values range from 0.58 to 0.61, in the range of 0.45 < n < 0.89, which corresponds to non-Fickian diffusion. Therefore, both water diffusion and polymer network relaxation will control the overall rate of swelling. The diffusion constant k, which incorporates structural characteristics of the hydrogels, is a relaxation rate. Both the n and k values of hydrogels with lower crosslinking ratios, that is, G-EGDE-1 and G-P200-1, are larger than those having higher crosslinking ratios, that is, G-EGDE-3 and G-P200-3. Lower crosslinking will result in more pronounced polymer network relaxation, thus inducing higher swelling efficiency.

Table 1. Recovery Ratio R (%) of the (1 → 6)-α-D-Glucan Hydrogels and Parameters of the Peppas Kinetic Equation, k and n

| Samples      | R (%) | k   | n    |
|--------------|-------|-----|------|
| G-EGDE-1     | 98.3  | 0.030 | 0.586 |
| G-EGDE-3     | 98.9  | 0.025 | 0.580 |
| G-P200-1     | 99.0  | 0.029 | 0.613 |
| G-P200-3     | 98.5  | 0.028 | 0.564 |

CONCLUSIONS

We synthesized the branchless (1 → 6)-α-D-glucan from sucrose using a recombinant GtfK enzyme from S. salivarius (ATCC 25975). The enzyme synthesis proceeded efficiently with a high yield of 80% under mild conditions. The branchless structure was water insoluble, unlike the widely available dextran with 5% branching. These advantages of the synthesis process and the unique structure and properties of the product suggest that it could be used to prepare functional materials from (1 → 6)-α-D-glucan.

As a demonstration of a functional material comprising branchless (1 → 6)-α-D-glucan, we prepared hydrogels with diglycidyl ethers as crosslinkers. The transparent and highly swellable hydrogels were much softer than other polysaccharide-based hydrogels with the same solid content. The compression tests of the hydrogels revealed several valuable properties: no syneresis, incompressibility, and complete shape recovery after repeated compression. The original size and shape of the hydrogels could also be recovered after...
dehydration by oven-drying. Because (1 → 6)-α-D-glucan is known to be degraded by dextran-1,6-glucosidase and dextranase in the human body,1,2 this hydrogel may have potential as a biomaterial in applications such as wound dressings, hemostasis, humectants, and drug delivery materials. Future studies should evaluate the biocompatibility and biodegradability of this hydrogel in the human body.

**EXPERIMENTAL SECTION**

**Preparation of the Recombinant Enzyme.** The *gftK* gene (Z11872.1) of *S. salivarius* (ATCC 25975) was cloned into a pET-21a(+) vector (Novagen, Madison, WI, USA). *Escherichia coli* (BL21-Gold (DE3) (Stratagene, La Jolla, CA, USA) introduced with the vector was incubated in lysogenic broth medium with 100 μg/mL of ampicillin and 1 mM of isopropyl β-D-1-thiogalactopyranoside at 37 °C. The recombinant GtfK enzyme was purified from the cell-crushed liquid of *E. coli* using the immobilized metal affinity chromatography method, as described in a previous work.26

To determine the optimal pH for the GtfK reaction, the purified GtfK solution was diluted to 1/20 volume and incubated at 37 °C in solutions with final sucrose and citrate phosphate (buffer) concentrations of 100 and 50 mM, respectively, and pH values between 3 and 9. After 30 min incubation, the enzyme was inactivated in a water bath at 90 °C for 3 min. The amount of fructose produced by the GtfK transfer reaction (Scheme 1) was measured by UV–vis spectrophotometry at 340 nm with enzyme kits (d-glucose/d-fructose UV-test, R Biopharm AG, Darmstadt, Germany). Because the optimal pH of the GtfK is 6.0 (Figure S1), the enzymatic reaction was carried out at pH 6.0 throughout this study.

**Synthesis of (1 → 6)-α-D-Glucan.** Purified GtfK (0.05 U/mL) was incubated for 3–7 days at 37 °C in 50 mM phosphate buffer (pH 6.0) containing 400 mM sucrose and 0.01% NaN₃. This was followed by the collection of samples were obtained using a 500 MHz NMR spectrometer (31278). FT-IR Spectroscopy.

**Solution NMR.** The (1 → 6)-α-D-glucan (40 mg) was dissolved in 1 mL of 2% (w/v) LiCl/dimethyl sulfoxide-d₆ with tetramethylsilane. One-dimensional (¹H and ¹³C) and two-dimensional (¹H–¹H COSY, ¹H–¹³C HSQC, and ¹H–¹³C HMBC spectroscopy) NMR spectra of the synthesized glucans were obtained using a 500 MHz NMR spectrometer (VARIANT, USA) at 60 °C. The chemical shifts of the assigned ¹H and ¹³C NMR peaks are as follows: ¹H NMR (δ, ppm): 4.70 (H1), 3.29 (H2), 3.50 (H3), 3.23 (H4), 3.67 (HS), 3.77 (H6a), 3.52 (H6b), 4.47 (C2OH), 4.92 (C3OH), 4.92 (C4OH). ¹³C NMR (δ, ppm): 98.13 (C1), 71.72 (C2), 73.41 (C3), 73.41 (C4), 70.32 (C5), 70.23 (C6), 66.19 (C6). FT-IR Spectroscopy. Freeze-dried samples of (1 → 6)-α-D-glucan and its hydrogels were ground with potassium bromide and pressed into disks. FT-IR spectra were measured in the absorbance mode using a spectrometer (IRPrestige-21, Shimadzu, Japan). Measurements were carried out between 4000 and 400 cm⁻¹ at a mirror speed of 2.8 mm/s, and 64 scans were collected at a resolution of 0.5 cm⁻¹.

**X-ray Diffraction.** (1 → 6)-α-D-glucan (1 g, Mₚ = 2.3×10⁵, Dₚ = 2.6) was hydrolyzed in 100 mL of 0.1 M HCl at 95 °C for 3 h. The residue was washed with deionized water and freeze-dried. The hydrolyzed (1 → 6)-α-D-glucan (yield = 80%, Mₚ = 6300, Dₚ = 1.3) was further oven-dried at 105 °C for 24 h (dry sample) or stored in a desiccator with a saturated KCl solution at 25 °C (RH = 85%) (wet sample). The dry and

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wet samples were filled in the sample holder, gently pressed to obtain a smooth surface, and set in the goniometer of an X-ray diffractometer (Ultima IV, Rigaku, Japan). For the wet sample, a container with water was placed in the sample chamber to prevent drying. XRD in the reflection mode was performed using Cu Kα radiation (λ = 0.15418 nm). The XRD patterns were collected between 2θ of 5 and 36° at a scanning rate of 0.8°/min.

**Solid-State CP/MAS ^{13}C-NMR.** Solid-state CP/MAS ^{13}C NMR spectra of (1 → 6)-α-D-glucan and the freeze-dried hydrogels were recorded using a 400 MHz solid-state NMR spectrometer (VARIAN, USA) using dipolar decoupling with a 4.0 mm double resonance probe. The dry and wet samples of (1 → 6)-α-D-glucan and freeze-dried hydrogels were packed uniformly in a zirconia rotor. The rotor was spun at 15 kHz using a 3.0 μs proton excitation pulse, a CP contact time of 2.0 ms, scanning of 2048 times, and relaxation delay of 5 s.

**Optical Transmittance.** The optical transmittance of (1 → 6)-α-D-glucan hydrogels was investigated using an UV–Vis spectrophotometer (UV-2450, Shimadzu, Japan). The spectra between 300 and 900 nm were recorded using the hydrogel with 3.0 mm thickness.

**Mechanical Properties.** Compression tests were carried out in a thermostatic chamber (25 °C, RH = 50%) using a testing machine (EZ-Test, Shimadzu, Japan) with a 100 N load cell. Cylindrical (1 → 6)-α-D-glucan hydrogels of 10–15 mm in diameter and 15–15 mm in height were compressed with a measuring plate at a constant speed of 1 mm/min. Five measurements were performed for each condition. The elastic modulus was determined from the linear region of the stress–strain curve. The photographs of the compression tests were taken using an EOS Kiss X10 (CANON, Japan) camera, and the height and diameter of the hydrogels were determined using Photoshop (ADOBE, USA). Poisson’s ratio, ν, was calculated using the following equation

\[ ν = -\frac{ε_ε}{ε_h} \]

where ε_ε and ε_h are strain of cylindrical hydrogels along the height and diameter directions, respectively.

**Water Swelling Kinetics.** The kinetics of water swelling of the (1 → 6)-α-D-glucan hydrogels from oven-dried samples was measured. The (1 → 6)-α-D-glucan hydrogels were dried at 60 °C for 24 h. The dried samples weighing about 30 mg were immersed in deionized water at room temperature. The hydrogel was taken out of the deionized water, and the weight of the hydrogel was measured at specific times after wiping excess water from the surface. The first 60% of the water swelling data were fitted by the Peppas kinetic equation

\[ \frac{M_t}{M_e} = k t^n \]

where \( M_t \) and \( M_e \) are the mass of water absorbed at time \( t \) and at equilibrium (after immersing in water for 3 days), respectively; \( M_t/M_e \) is the water uptake; \( k \) is the diffusion constant related to the relaxation rate of the crosslinked structure; and \( n \) is the diffusion exponent indication of transport mechanisms. For cylindrical samples, \( n < 0.45, n = 0.45, \) and \( 0.45 < n < 0.89 \) indicate less-Fickian, Fickian, and non-Fickian diffusion, respectively.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04699.

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**Notes**

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