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Bio-Based Hydrogels Prepared by Cross-Linking of Microbial Poly(γ-glutamic acid) with Various Saccharides

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Novel bio-based hydrogels were prepared by cross-linking of microbial poly(γ-glutamic acid) (PGA) with saccharides such as glucose, maltotriose, and cyclodextrin (CD) in the presence of water-soluble carbodiimide in dimethyl sulfoxide (DMSO) by one-pot synthesis at 25 °C for 24 h. The degradation of the gels in alkaline solution (pH 9) at 37 °C was also investigated. The PGA gels cross-linked with various neutral saccharides were obtained in relatively high recovery yields by use of a base like 4,4-(dimethylamino)pyridine. The PGA gel cross-linked by glucose showed the highest water absorption of 3000 g/g. The PGA gels cross-linked by CDs showed higher water absorption than those cross-linked by the corresponding linear saccharides. It was revealed that the water absorption of the PGA gel was affected by the cross-linker content and also the structure of cross-linkers as they had an effect on the cross-linking density of the PGA gel. The PGA gels were hydrolyzed under alkaline condition (pH 9) at 37 °C. The degradation rate was higher when the cross-linker content of the gel was lower.

Introduction

Poly(γ-glutamic acid) (PGA) is a polymer produced by some microorganisms such as *Bacillus subtilis* F-2-01[1] and *Bacillus subtilis* IFO3335.[2] PGA is biodegradable and biocompatible, so that many researchers have reported the modification of PGA. For example, the esterification of PGA was studied for pharmaceutical applications.[3] In addition, cross-linked PGA gels were prepared by use of γ-irradiation[4−7] or cross-linking reagents, such as dihalogenoalkanes[8,9] and alkyldiamines.[10] Some of these gels could absorb a large amount of water and their applications were mainly for the medical-related or environmental area, such as a drug-release system[5,6,8−10] and water treatment reagents.[7] However, the cross-linkers used in most of the studies were compounds of petroleum origin. Considering that biodegradable gels will be degraded in the environment or in human bodies after use, it is important not only that the main chains of the gel are naturally occurring polymers but also that the cross-linkers should be natural compounds. And materials derived from biomass resources are required for reduction of CO_2 release or preservation of petroleum.

We now report novel bio-based hydrogels prepared by cross-linking of PGA with saccharides by esterification in the presence of a water-soluble carbodiimide (WSC) in dimethyl sulfoxide (DMSO) by one-pot synthesis without any modification of substrates. Kunioka and Furusawa[10] have reported the cross-linking of PGA with alkyldiamines by amidation in the presence of WSC in aqueous solution, and the highest yield was 39.9 mg of PGA gel from 100 mg of PGA. In this article, the PGA gels cross-linked by saccharides were obtained in higher recovery yields in DMSO solution compared to the yields in aqueous solution. The alkaline hydrolysis of the gels was also investigated.

Experimental Section

Materials and Measurements. PGA was kindly gifted from Meiji Seika Kaisha, Ltd. (Tokyo, Japan). The molecular weight of the PGA was 3.1×10^5 from the product data provided by Meiji Seika Kaisha, Ltd. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC), D-(+)-glucose, D-(−)-fructose, and lactose monohydrate were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Water-soluble chitosan (deacetylated chitin), maltotriose, maltopentaose, maltohexaose, maltoheptaose, α-, β-, and γ-cyclodextrin (α-, β-, and γ-CD, respectively), 4,4-(dimethylamino)pyridine (DMAP), and 4-pyroridinopyridine (4-PP) were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). The weight-average molecular weight (M_w) of water-soluble chitosan was measured by size-exclusion chromatography (SEC) on a SEC column (Waters Protein-Pak 125SW, Waters Corp., Milford, MA). The system was calibrated with protein standards. Water-soluble chitosan showed a wide range of molecular weights and the M_w was 9.7×10^5. Degree of deacetylation was 0.61 by elemental analysis. DMSO was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). All reagents were reagent-grade and were used as received.

To estimate the amount of cross-linkers contained in the PGA gels, the ^{1}H NMR spectra of the hydrolysates of the PGA gels were recorded on an FT-NMR spectrometer JNM-A400 (JEOL, Ltd., Tokyo, Japan) operating at 400 MHz. A signal of water at 4.80 ppm (from 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DDS)) was used as an internal reference. The insoluble PGA gel was placed in a test tube and then D_2O was added. The pH of the solution was adjusted to 10 with NaOD/D_2O solution. The mixture stood overnight at 60 °C. The PGA gel dissolved gradually as the hydrolysis proceeded. Then the NMR spectra of the solution of the PGA gel were measured.

The IR spectra were measured by FTIR-8200PC (Shimadzu Corp., Kyoto, Japan). The samples were pelletized with KBr (Jasco Corp., Tokyo, Japan) for the measurement.

The hydrolysis of the PGA gel under alkaline conditions was monitored by SEC (Waters 650 system) on SEC columns (Waters Protein-Pak 300SW × 2, Waters Corp., Milford, MA). PGA was detected at 220 nm with a Waters 486 tunable absorbance detector. Tris-HCl aqueous solution (50 mM, pH 7.5) was used as an eluent at 0.50 mL min^{-1}.

Cross-Linking of PGA and Neutral Saccharides in DMSO by Esterification. The method to prepare the PGA gel is shown in Scheme

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Scheme 1. Cross-Linking of PGA by Neutral Saccharides

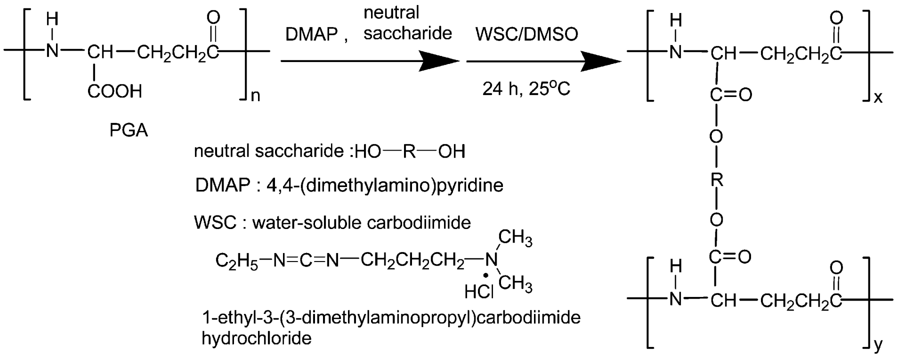


Table 1. Poly(γ-glutamic acid) Gel Prepared from PGA and 1,6-Hexanediol by Use of Water-Soluble Carbodiimide and Various Bases in DMSO[a]

	base	amount (mg)	molar ratio of base to Glu residue of PGA (mol %)	recovered wt (mg)	recovery yield (%)
a	$NaHCO_3$	32.5	50	trace	
b	triethylene-diamine	8.7	10	trace	
c	DMAP	9.4	10	123.6	100
d	4-PP	11.4	10	116.2	94.5

[a] Reaction conditions: 100 mg of PGA, 80 mg of WSC, 22.9 mg of 1,6-hexanediol, and 1.0 mL of DMSO; 25 °C for 24 h.

Table 2. PGA Gel Prepared from PGA and Various Saccharides[a]

cross-linker	(mg)	solvent	recovered wt (mg)	recovery yield (%)	water absorption (g/g)
D-fructose[b]	34.9	DMSO	131.3	97.4	354
lactose[b]	69.8	DMSO	147.0	86.6	387
α-CD[b]	188.4	DMSO	163.6	56.7	882
β-CD[b]	218.5	DMSO	100.0	31.4	933
γ-CD[b]	251.1	DMSO	155.5	44.3	981
water-soluble chitosan[c]	15.6[d]	DMSO	46.5	70.9	938
water-soluble chitosan[e]	15.6[d]	1.0 M NaOH	17.0	23.8	1208

[a] Various saccharides (25 mol %) were added to the glutamic acid residue of added PGA by use of water-soluble carbodiimide at 25 °C for 24 h. [b] Reaction conditions: 100 mg of PGA, 80 mg of WSC, 9.4 mg of DMAP, and 1.0 mL of DMSO. [c] Reaction conditions: 50 mg of PGA, 40 mg of WSC, and 0.50 mL of DMSO. [d] Water-soluble chitosan was added as 0.08 mL of aqueous solution. [e] Reaction conditions: 50 mg of PGA, 40 mg of WSC, and 0.19 mL of 1.0 M NaOH.

1. A 100 mg (0.77 mmol) sample of PGA, 9.4 mg (0.077 mmol) of DMAP, and saccharides were put into a glass test tube, and then 0.70 mL of DMSO was added. The contents of the tube were dissolved in DMSO by ultrasonication. The DMSO solution of WSC was added to the reaction solution, and the solution was stirred at 25 °C. After 24 h, an excess amount of acetone was added to the test tube and the reaction product was isolated as a precipitate by decantation. The product was then kept in a pH 7 phosphate buffer solution overnight, and 1.0 M NaOH was added to adjust the pH of the solution to 8. The solution containing the swelled PGA gel was put into a bag made of nylon mesh (mesh 255, 57 μm open pitch) and filtered off. The PGA gel was washed by dipping the bag in distilled water and changing the water once a day for a week. After removal of the excess water, the swelled gel was lyophilized to obtain the dry PGA gel. Recovery yields of the dry PGA gel were calculated from the following equation:

$$\text{recovery yield (\%)} = W_{\text{gel}}/(W_{\text{PGA}} + W_{\text{CL}}) \times 100 \qquad (1)$$

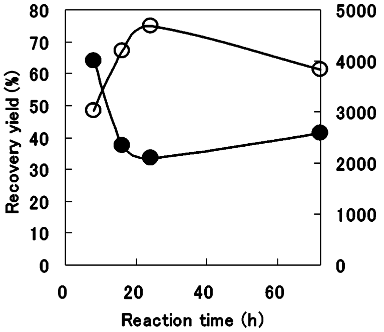


Figure 1. Time course of recovery yield and water absorption of the obtained poly(γ-glutamic acid) (PGA) gel. The PGA gels were prepared by cross-linking PGA with β-cyclodextrin (β-CD) in DMSO at 25 °C by use of 100 mg of PGA, 43.6 mg of β-CD, 80 mg of water-soluble carbodiimide (WSC), 9.4 mg of 4,4-(dimethylamino)pyridine (DMAP), and 1.0 mL of DMSO. Recovery yield (○) and water absorption (●) are shown.

where W_{gel} is the weight of the obtained dry PGA gel, W_{PGA} is the weight of the dry PGA before cross-linking, and W_{CL} is the weight of a cross-linker used for the reaction

Cross-Linking of PGA by Water-Soluble Chitosan. The PGA was placed in a test tube and dissolved in DMSO by ultrasonication. Just after the DMSO solution of WSC was added, an aqueous solution of water-soluble chitosan was added to the reaction mixture and it was stirred at 25 °C for 24 h. The obtained PGA gel was washed and dried by the method described above. No additional base was added when the water-soluble chitosan was used as a cross-linker. In the case of the reaction in water, the PGA was dissolved in 1.0 M NaOH and then an aqueous solution of water-soluble chitosan and WSC were mixed in the PGA solution in order.

Water Absorption of PGA Gels. The dried PGA gels were weighed and put into a nylon mesh bag. The bag was then dipped in distilled water. After 24 h, it was hung for 10 min to remove the excess water and weighed. The same operation was carried out with an empty nylon bag as the blank. The water absorption (gram per gram) of the PGA gel was calculated from the following equation:

$$\text{water absorption} = (Q_1 - Q_0)/Q_0 \qquad (2)$$

where Q_0 is the weight of the dry PGA gel and Q_1 is the weight of the swelled PGA gel.

Alkaline Hydrolysis of PGA Gels. Several samples of the PGA gels were hydrolyzed at pH 9 in buffered solution (0.1 M boric acid−borax) at 37 °C. Each PGA gel was added to the buffer at a concentration of 2 mg/mL. The hydrolysis was periodically monitored by SEC analysis of the supernatants.

Results and Disscussion

Cross-Linking of PGA by Diols, Neutral Saccharides, and Water-Soluble Chitosan. For the cross-linking of PGA with

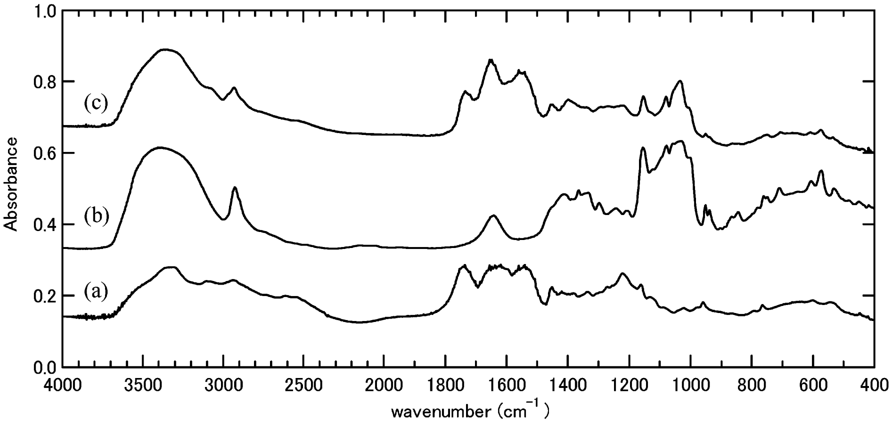


Figure 2. IR spectra of (a) PGA, (b) α-CD, and (c) PGA gel cross-linked by 25 mol % α-CD to the glutamic acid residue.

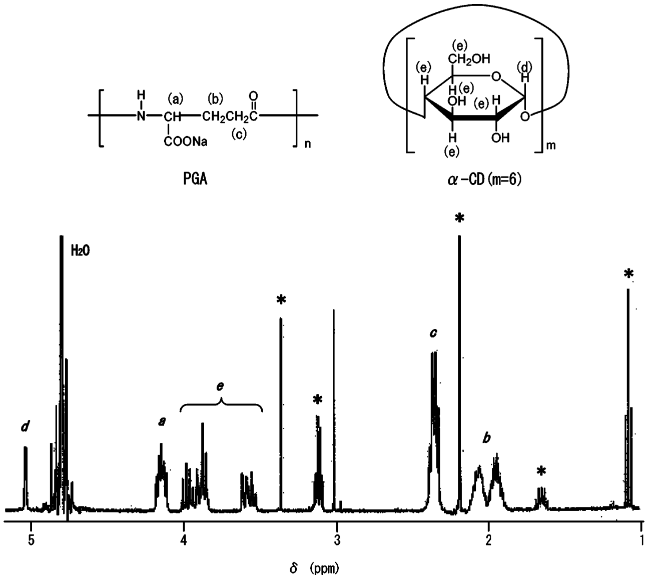


Figure 3. ^{1}H NMR spectrum (400 MHz) of hydrolysates of PGA gel cross-linked by α-CD in D_2O. A signal of water at 4.80 ppm (from DSS) was used as an internal reference. The PGA gel was stood overnight at 60 °C to dissolve in pH 10 NaOD/D_2O solution. *N-Acyl urea produced after the addition of α-CD or the PGA-WSC adduct remained in the gel (Scheme 2).

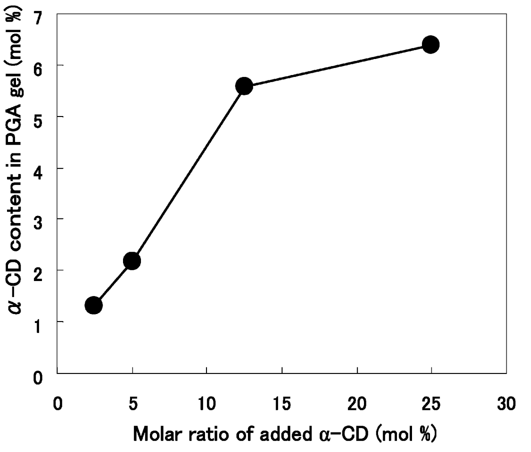


Figure 4. Relationship between molar ratio of α-CD added to the reaction solution and α-CD content in PGA gel calculated from NMR spectra. The PGA gels were prepared with 100 mg of PGA, 9.4 mg of DMAP, 80 mg of WSC, and 1.0 mL of DMSO at 25 °C for 24 h.

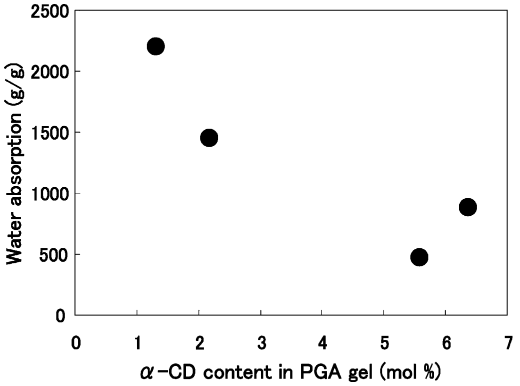
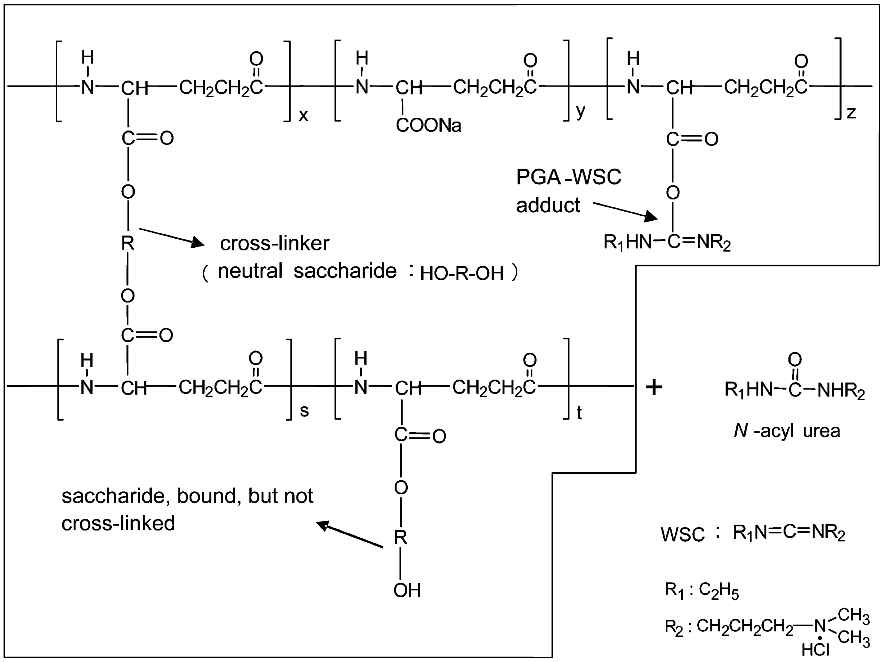


Figure 5. Relationship between water absorption and α-CD content in PGA gels. The PGA gels were prepared with 100 mg of PGA, 9.4 mg of DMAP, 80 mg of WSC, and 1.0 mL of DMSO at 25 °C for 24 h.

neutral saccharides, the PGA must be esterified with the saccharides (Scheme 1). To find the appropriate condition for the esterification of PGA, preliminary experiments were carried out with 1,6-hexanediol as the cross-linker. As a result, it was revealed that the cross-linking promoted by WSC did not occur in water, but it successfully proceeded in DMSO in the presence of a base (Table 1). 1,6-Hexanediol was added at 25 mol % to the glutamic acid residue of PGA. When DMAP or 4-PP was added, the PGA gel was obtained with a high recovery yield. The recovery yield in the case of DMAP exceeded 100% and was slightly higher than that in the case of 4-PP. The yield was calculated by eq 1 with the weight of the un-cross-linked PGA with the free carboxyl acid groups as part of the denominator. The apparent recovery yield larger than 100% probably resulted because the obtained PGA gels became sodium salts after washing. On the basis of the preliminary experiments, the cross-linking of PGA with neutral saccharides was investigated with WSC and DMAP in DMSO, and the results are shown in Table 2. The reactions were carried out at 25 °C because the gelation had not occurred over 25 °C in the preliminary experiments. PGA gels were obtained with various saccharides, such as D-(−)-fructose (monosaccharide), lactose (disaccharide), α-, *β*-, and *γ*-CD (cyclic oligosaccharides), and water-soluble chitosan (amino sugar). The cross-linking by neutral saccharides was carried out with 0.77 mmol (100 mg) of PGA, 25 mol % saccharides to the glutamic acid residue of PGA, 54 mol % (80 mg) WSC, 0.10 mol % (9.4 mg) DMAP, and 1.0 mL of DMSO in total (0.77 mol/L of PGA concentration). As for the cross-linking by water-soluble chitosan in DMSO, the smaller amount of PGA (50 mg) was used in order to mix the contents homogeneously with the water-soluble chitosan aqueous solution. PGA (3.9 mmol, 50 mg), 54 mol % WSC (40 mg) and 0.50 mL of DMSO (0.77 mol/L of PGA concentration) were mixed with 25 mol % water-soluble chitosan (15.6 mg)

Scheme 2. Possible Structure of PGA Gels Cross-linked by Neutral Saccharides



dissolved in 0.08 mL of water. The molar ratio of each reagent was the same amount as that used for the cross-linking by neutral saccharides. As for the cross-linking in aqueous solution, the PGA gel was prepared from 3.9 mmol (50 mg) of PGA and 25 mol % water-soluble chitosan (15.6 mg) dissolved in 0.27 mL of water with 54 mol % WSC (40 mg). High water absorption was observed when the recovery yield of the gel was low, regardless of the kind of the saccharide. The PGA gel obtained in the lower recovery yield might be cross-linked by a lower amount of saccharide. The decrease in the amount of bound saccharide meant a decrease in the cross-linking density of the gel and it caused an increase of water absorption. The effects of the structure and the amount of various saccharides on the recovery yields and water absorption of the PGA gels will be discussed later.

The time course of the recovery yield in the case of cross-linking of PGA by 5.0 mol % β-CD to the glutamic acid residue and water absorption of the obtained PGA gels was plotted in Figure 1. The water absorption decreased with increasing recovery yield up to 24 h. This was attributed to the increase in the cross-linking density with the increasing β-CD binding to PGA as discussed above. However, after 72 h, the recovery yield decreased and the water absorption increased. This might be caused by cleavage of a part of the cross-linking of the gel after the reaction mixture stood for a long time. The highest yield in Figure 1 was 75% (108 mg of PGA gel from 100 mg of PGA), which was much higher than the yields previously reported in the corresponding cross-linking of PGA by diamines with WSC in aqueous solution at room temperature.[10] The IR spectra of PGA, α-CD, and the PGA gel cross-linked by α-CD are shown in Figure 2. On the IR spectrum of the PGA gel, several characteristic peaks from α-CD, such as 1157, 1084, and 1028 cm^{-1}, appeared in addition to the peaks due to PGA. This proved that the PGA gel contained α-CD moieties.

For the cross-linking of PGA by water-soluble chitosan, the chitosan was added as an aqueous solution because it is insoluble in DMSO. It should be noted that this reaction did not need any additional base like DMAP because chitosan itself has active amino groups. The recovery yield, 70.9%, was comparable with the results of the other saccharides. As shown in Table 2, the PGA was also cross-linked by the chitosan in aqueous solution as well as in DMSO because amide bonds could be formed in water in the presence of WSC.[10] However, the recovery yield in PGA aqueous solution, 23.8%, was lower than that in DMSO. The low recovery yield in the aqueous solution suggested that more WSC was deactivated in water than in DMSO. This reaction method for the water-soluble chitosan is available for cross-linking PGA by use of basic amino acids such as L-lysine or L-ornithine.[11]

Effect of the Amount of Added Cross-Linker on the Cross-Linker Content in PGA Gels and Water Absorption. The ^{1}H NMR spectrum of the solution of the hydrolysate of the PGA gel cross-linked by α-CD is shown in Figure 3. The α-CD contents in the gels (mole percent) were estimated from the following equation:

$$Y = [A_{6H}/(6/m)]/A_{PGA} \qquad (3)$$

where Y is the molar ratio of saccharides to the glutamic acid residue of PGA, A_{6H} is the peak area of protons (H-2,-3, -4, -5, -6, and -6′) of glucose residues, m is the number of the glucose residues of saccharides ($m = 6$ in the case of α-CD or maltohexaose), and A_{PGA} is the peak area of the α-proton of the glutamic acid residue of PGA.

To investigate the effect of the amount of an added cross-linker on the cross-linker content in the PGA gels and the water absorption, several PGA gel samples were prepared with different molar ratios of α-CD to the glutamic acid residue of added PGA. Figure 4 shows that the α-CD content in the PGA gel increased with the amount of α-CD added to the reaction solution. The α-CD contents were estimated from the ^{1}H NMR spectra by use of eq 3. If α-CD acts as a bifunctional cross-linker, all of the α-CD theoretically bind to PGA under the reaction conditions stated in Figure 4. However, the reactivity of the added α-CD, estimated from the α-CD content of the PGA gel in Figure 4, decreased with increasing molar ratios of α-CD added to the reaction. The low reactivity was considered to be due to the steric hindrance of α-CD. The bulky structure of α-CD might interfere with the α-CDs binding to the carboxyl

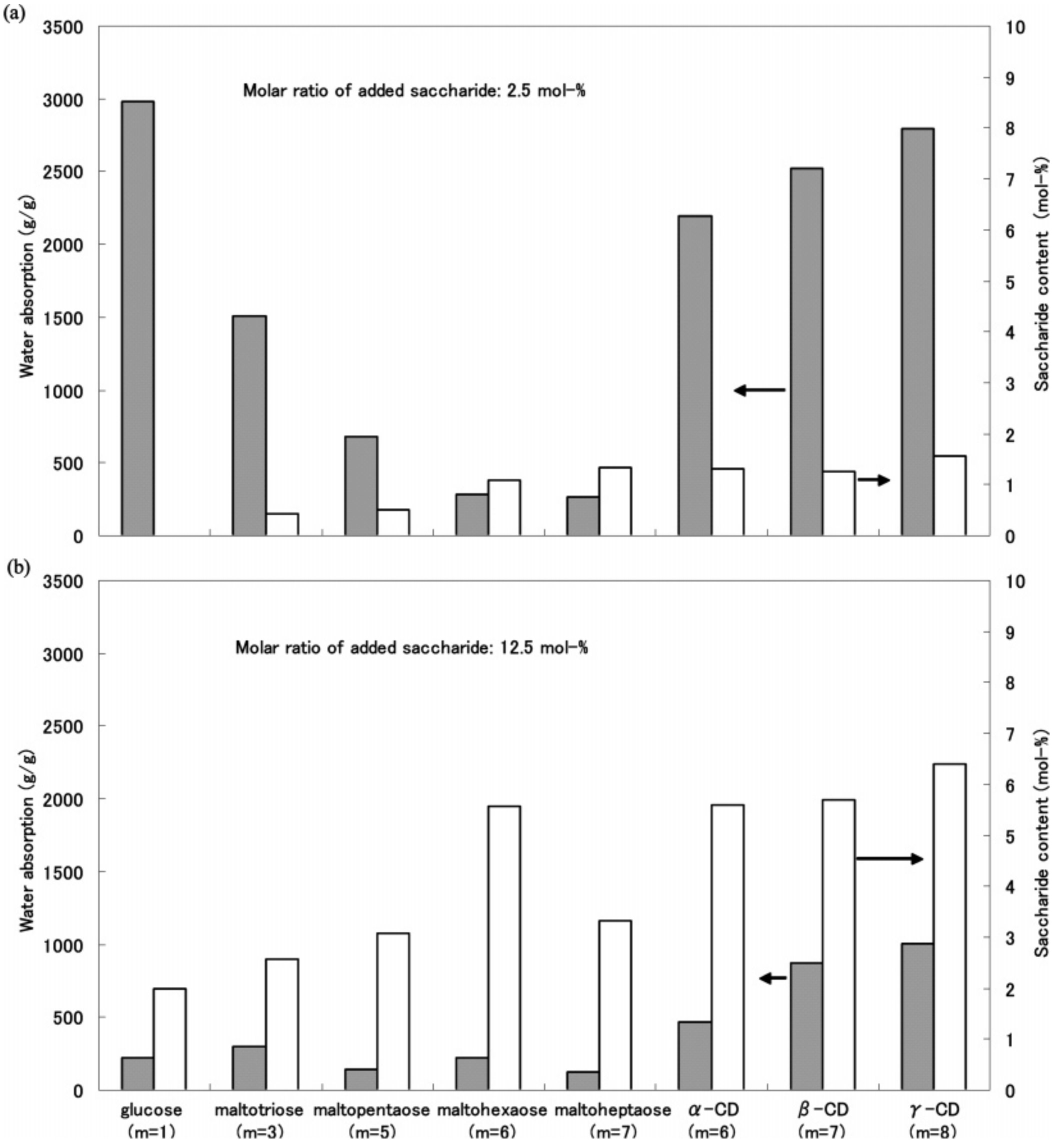


Figure 6. Water absorption (left gray bars) and saccharide content (right open bars) of PGA gels cross-linked by saccharides, by use of 100 mg of PGA, 9.4 mg of DMAP, 80 mg of WSC, and 1.0 mL of DMSO at 25 °C for 24 h. The molar ratio of saccharides, used for the cross-linking, to the glutamic acid residue of PGA was 2.5 mol % (a) and 12.5 mol % (b).

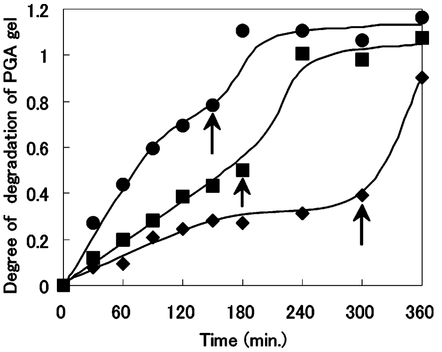


Figure 7. Alkaline hydrolysis profiles of PGA gels cross-linked by α-CD. The α-CD contents of the PGA gels were 1.3 mol % (●), 2.2 mol % (■), and 5.6 mol % (◆).

groups activated by WSC; thus the reactivity of α-CD in the gel decreased when the molar ratio of α-CD used for the reaction was higher.

Figure 5 shows the relationship between water absorption and α-CD content in the PGA gel estimated by the NMR spectra. The water absorption increased from 470 to 2200 g/g as the α-CD content in the PGA gel decreased from 5.6 to 1.3 mol %. This result is probably due to the fact that the cross-linking density of the PGA gel became lower with lower α-CD content. However, when the α-CD content was 6.4 mol %, the PGA gel had higher water absorption than when the α-CD content was 5.6 mol %. The PGA gel containing 6.4 mol % α-CD was obtained with 25 mol % α-CD, which was the highest amount in Figure 5. When the large amount of α-CD was used, some of the α-CD did not cross-link with the PGA molecules and bound to the PGA as a branched side chain, as indicated in Scheme 2. As a result, the cross-linking density of the PGA gel with 6.4 mol % α-CD became somewhat low; therefore, the water absorption increased more than the PGA gel with 5.6 mol % α-CD.

Effect of Cross-Linker Structure on Water Absorption of PGA Gels. The water absorption and cross-linker contents of the PGA gels cross-linked by various saccharides consisting of glucose residues were compared (Figure 6). The saccharide contents in the PGA gels were determined by eq 3. The glucose content in Figure 6a was not shown because the proton peaks of glucose on the ^{1}H NMR spectrum were too small for calculation.

The cross-linking reactions were conducted with 2.5 or 12.5 mol % saccharides to the glutamic acid residue of PGA. These results are shown in Figure 6, panels a and b, respectively.

The water absorptions in Figure 6a were higher than those in Figure 6b because the low saccharide contents of the gels in Figure 6a made the network coarser than that of the gels in Figure 6b. This indicates that the cross-linker contents have effects on both the cross-linking density and water absorption of the gels.

Cyclic saccharides α-CD ($m = 6$) and β-CD ($m = 7$) have the same number of glucose residues as linear saccharides maltohexaose ($m = 6$) and maltoheptaose ($m = 7$), respectively. In Figure 6a, the saccharide contents of maltohexaose, maltoheptaose, and the CDs were almost equal. However, the gels with the CDs showed higher water absorption than the gels with maltohexaose and maltoheptaose. This result may be explained by the difference between the cyclic structure of the CDs and the linear structure of maltohexaose or maltoheptaose. First, the bulky structure of the CDs might interfere with the cross-linking of PGA by the CDs and make the network of the gel coarser, so that the gels with the CDs showed high water absorption. Second, the probability of collision between the linear saccharide bound to one position of the PGA chain and the other position of the PGA chain may be higher than that between the bound cyclic CD and the PGA chain. And this is probably the cause of the higher cross-linking density, which resulted in lower water absorption, of the PGA gels cross-linked by the linear saccharides, especially for the larger oligosaccharides such as maltohexaose or maltoheptaose in Figure 6.

Alkaline Hydrolysis of PGA Gels. The degradation test was conducted with PGA gels cross-linked by α-CD in alkaline solution (pH 9) at 37 °C. The α-CD contents of the samples were 1.3, 2.2, and 5.6 mol %. The water absorption values of these samples were 2200, 1450, and 470 g/g, respectively. After several hours, PGA was dissolved in the alkaline solution. The SEC analysis indicated that the dissolved PGA had almost the same molecular weight as that of the PGA before cross-linking. This means that dissolution was due to cleavage of the cross-linking and not of the PGA main chain. The concentration of PGA in the supernatant was periodically monitored by SEC analysis with the 2.0 mg/mL PGA solution as a standard. The degree of degradation of the cross-linked gels, shown in Figure 7, was estimated from the following equation:

$$D_{\mathrm{gel}} = 2(S_{\mathrm{gel}}/S_{\mathrm{PGA}})/C_{\mathrm{gel}} \qquad (4)$$

where D_{gel} is the degree of degradation of the PGA gel, C_{gel} is the maximum concentration of PGA in the case of 100% degradation of the gel, S_{PGA} is the peak area of 2 mg/mL PGA aqueous solution as a standard, and S_{gel} is the peak area of PGA released from the gel to the supernatant.

The maximum concentrations (C_{gel}) in the case of 100% degradation, estimated roughly from the ^{1}H NMR spectra, were 1.8, 1.7, and 1.4 mg/mL for the PGA gels with 1.3, 2.2, and 5.6 mol % α-CD content, respectively. Ester linkages in the cross-linking structure of PGA and α-CD were gradually hydrolyzed and the concentrations of the dissolved PGA were increased. It took a longer time for the PGA gel with higher α-CD content to dissolve in the solution up to 100%. The reason that some of the degrees of degradation exceeded 1 was not obvious at present, but it might be caused by fluctuation of SEC measurements.

The specific times when the concentration of PGA significantly increased were observed in Figure 7 as indicated by the arrows. The times for the PGA gels with 1.3, 2.2, and 5.6 mol % α-CD contents were 150, 180, and 300 min, respectively. The specific time appeared later for the PGA gel having the higher α-CD content because the PGA gel with the higher α-CD content had more ester linkages to be hydrolyzed for dissolution. The time is considered to be a kind of critical time when the hydrolysis rate was accelerated due to dissolution of the gel. The PGA molecules near the surface of the gel were released and observed in the supernatant on the first stage of the degradation. And on this stage the PGA molecules inside the gel were trapped in the cross-linking network. At the specific time the network was collapsed and PGA molecules of which cross-linking points had been hydrolyzed enough to be soluble were freely released to the supernatant.

Conclusions

Novel bio-based hydrogels were prepared by cross-linking of microbial PGA with various saccharides in the presence of WSC in DMSO. The PGA gels cross-linked by CDs showed higher water absorption than those cross-linked by other saccharides. It was revealed that water absorption of the PGA gel was affected by the cross-linker content and also the cross-linker structure. Both of these can have an effect on the cross-linking density of the PGA gel. The increase and decrease in the water absorption can be explained by the change in the cross-linking density of the PGA gels. The gels were hydrolyzed under alkaline conditions. The degradation rate was higher when the cross-linker content of the gel was lower. The gels obtained in this study were considered to be biodegradable because they consisted only of natural biodegradable compounds bound by ester linkages at cross-linking points.

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