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Preparation, Water Absorbency, and Enzyme Degradability of Novel Chitin- and Cellulose/Chitin-Based Superabsorbent Hydrogels

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ABSTRACT: Superabsorbent hydrogels were prepared from chitin dissolved in lithium chloride and *N*-methyl-2-pyrrolidinone by esterification crosslinking with 1,2,3,4-butanetetracarboxylic dianhydride (BTCA). The absorbency of the chitin hydrogel was strongly dependent on the ratio of BTCA feed to chitin. The hydrogel prepared at the feed ratio of 5 showed the highest absorbency (345 g/g-polymer), and the hydrogel was composed of 0.65 molecules of BTCA per monomer unit of chitin. The hydrogels exhibited good biodegradability by chitinase with a maximum degradation of 91% within 7 days. This method for obtaining the chitin hydrogel was also applicable to cellulose and chitin mixtures, and 1 : 1 cellulose/chitin hybrid hydrogels could be obtained by the esterification crosslinking of a mixture with a 1 : 1 molar ratio of cellulose and chitin. The optimal BTCA feed ratio of 5 resulted in the cellulose/chitin hydrogel with the highest water absorbency (329 g/g-polymer), and the hydrogel contained 0.65 molecules of BTCA per polysaccharide monomer unit. In addition, the hybrid hydrogels were degraded by cellulase as well as chitinase. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: biodegradable; spectroscopy; polysaccharides; swelling; crosslinking

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INTRODUCTION

Superabsorbent polymers (SAPs) are polymers that are capable of absorbing and retaining extremely large amounts of water.[1] The most common type of SAP used in the world today is crosslinked sodium polyacrylate (SPA), which is made from the copolymerization of acrylic acid and acrylic acid sodium salt.[2] SPA has the ability to absorb as much as 300–500 times its mass in water and thus, is extensively used in applications such as baby diapers, adult protective underwear, sanitary napkins, and horticultural soil additives. One of the biggest drawbacks of SPA is that it is nonbiodegradable despite its widespread use in the category of disposable goods. Thus, the development of biodegradable SAPs as substitutes for SPA is necessary for preventing environmental pollution associated with synthetic polymers.

Prime candidates as starting materials for biodegradable SAPs are cellulose and chitin, which are the first and second most abundant organic substances on earth, respectively. These polysaccharides show biocompatible and biodegradable properties, thus, they are of considerable interest in the development of environment friendly and biocompatible materials.[3–5] Structurally, cellulose consists of a straight chain of β-(1→4)-linked D-glucose residues (Glc). Chitin, which is structurally similar to cellulose, is a polymer composed of β-(1→4)-linked *N*-acetyl-D-glucosamine residues (GlcNAc), and some of the GlcNAc residues in the polymer chain are replaced by D-glucosamine residues (GlcN). Although considerable efforts are still being devoted to the development of novel applications of cellulose and chitin, including conversion to biodegradable SAPs as this represents the efficient use of biomass resources, the lack of solubility of these polysaccharides in water and common organic solvents presents challenges in improving their functionality. The solubility problem associated with these polysaccharides is owing to the molecular rigidity and close chain packing that result from numerous inter- and intramolecular hydrogen bonds caused by the hydroxyl groups in the structures of cellulose[6] and chitin.[7] To date, only a limited number of solvent systems for cellulose and chitin capable of disrupting the strong hydrogen bonding interactions have been found.[8–10] For example, lithium chloride (LiCl)/*N*-methyl-2-pyrrolidinone (NMP),[11] LiCl/*N*,*N*-dimethylacetamide,[12] and tetrabutylammonium fluoride (TBAF) trihydrate/dimethyl sulfoxide (DMSO)[13,14] are well-known as convenient solvents for these polysaccharides. The homogeneous reactions of cellulose and chitin using these solvent systems offer the advantages of creating options for incorporating functional groups, open new avenues for the

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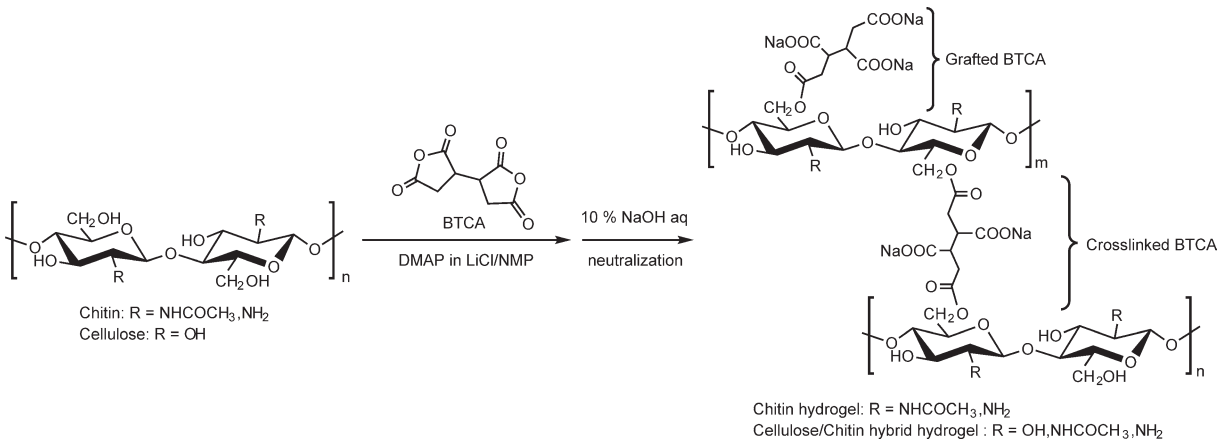

Scheme 1. Synthesis of chitin and cellulose/chitin hybrid hydrogels.

design of reaction products, broaden the opportunity to control the degree of substitution (DS), and so forth. Many reports on the chemical modification of cellulose and chitin in these solvent systems have been reported previously.[8,15,16]

Some methods for the conversion of cellulose or chitin to SAP in the homogenous reaction solvents have also been reported. For example, biodegradable SAP was obtained from cellulose and succinic anhydride in LiCl/NMP and TBAF/DMSO systems without any crosslinker when 4-dimethylaminopyridine (DMAP) was used as the reaction catalyst.[17] Other articles by the same research group reported that biodegradable hydrogels were further obtained from chitin,[18] starch, and guar gums[19] using experimental procedures similar to the preparation of the hydrogel of cellulose with succinic anhydride. We have also studied the preparation of superabsorbent hydrogels using cellulose dissolved in LiCl/NMP by esterification crosslinking with 1,2,3,4-butanetetracarboxylic dianhydride (BTCA)[20] because BTCA has two acid anhydrides in its structure, each of which reacts readily with certain functional groups such as isocyanate and hydroxyl to undergo crosslinking.[21] In the reaction of cellulose with BTCA, two free carboxylate groups were formed with simultaneous crosslinking of cellulose chains; hence, the absorbency of the product was expected to be enhanced in comparison with that of the succinylated cellulose hydrogel. As a result, the use of cotton cellulose produced a hydrogel with an absorbency of 720 times its dry weight, and the hydrogels exhibited good biodegradability with a maximum degradation of 95% within 7 days using cellulase.[20] Thus, the product obtained by the esterification crosslinking of BTCA demonstrated exceptional potential as a substitute for conventionally used SPA.

In this article, we report on the detailed study of new superabsorbent hydrogels from chitin and BTCA including preparation, characterization, and biodegradability by chitinase. In addition, to make hydrogels which can be easily degraded under diversified environmental conditions, biodegradable superabsorbent hydrogels have been prepared from the homogeneous mixture of cellulose and chitin dissolved in LiCl/NMP by a similar procedure for esterification crosslinking with BTCA. Preparation, characterization, and biodegradability of the cellulose/chitin hybrid hydrogels are also described herein.

EXPERIMENTAL

Materials

Powdered α-chitin (degree of acetylation [DA] = 82%, viscosimetric average molecular weight (M_W) = 5.4 × 10^5 g/mol, purchased from Tokyo Kasei Kogyo, Japan) and high-purity hardwood pulp (Sulfate HJ from Rayonier, M_W = 1.3 × 10^5 g/mol) were used for the starting materials without purification. BTCA and SPA were kindly supplied by Shin Nippon Rika, Japan, and Sundaiya Polymer, Japan, respectively. Sodium carboxymethylcellulose (CMC) with the DS of 0.72 was purchased from Junsei Chemicals, Japan. *Trichoderma viride* cellulase ONOZUKA R-10 and *Corynebacterium* sp. OZ-21 chitinase were purchased from Yakult Pharmaceutical Industry, Japan, and Takara BIO, Japan, respectively. All other reagents used in this study were analytical grade, which were purchased from Kanto Chemicals, Japan.

Preparation of Chitin Hydrogels

Superabsorbent hydrogels were prepared from chitin as shown in Scheme 1. As an example, the procedure for preparation of the hydrogel from chitin with the BTCA feed ratio of 1 is described in detail. DA of chitin used in this study was 82%, which indicates that 82% of all residues in the chitin were GlcNAc and that the remaining 16% were GlcN. Thus, the molecular mass of the chitin monomer was 188 g/mol, and this value was used to determine the molar mass of the chitin used in the reaction. Powdered chitin (1.0 g, 5.3 mmol for the monomer unit) was completely dissolved in an Erlenmeyer flask containing LiCl/NMP (5 g of LiCl and 95 g of NMP) under stirring with a Teflon impeller at 500 rpm at room temperature for 3 days, and then 0.64 g of DMAP (5.3 mmol) was added to the mixture. After complete dissolution of DMAP, 1.1 g of BTCA (5.3 mmol), which corresponds to a BTCA molar feed ratio of 1, was added to the mixture. Esterification was allowed to proceed with stirring at room temperature for 24 h, after which the reaction mixture was poured into methanol/water (1 : 1, 500mL) with stirring to precipitate the product. Next, the product was neutralized to pH 7.0 with 10% (w/v) aqueous NaOH by monitoring with a pH electrode. The precipitate was filtered using a glass filter, and was then purified twice by reprecipitation with methanol and water. The purified product was dried under reduced pressure, finely cut with a mixer, and

Table I. Reaction Conditions and Results of the Esterification Crosslinking of Chitin and a Cellulose/Chitin Mixture

Starting material	BTCA feed ratio[a]	I_{CH}[b]	I_{CH2}[b]	I_{CH3}[b]	n_{BTCA}[c]	Chitin composition[d] (mol %)	Cellulose composition[d] (mol %)	Absorbency[e] (g/g-polymer)
Chitin	1	0.17	0.16	0.80	0.08	100	0	125
	2.5	0.71	0.66	0.79	0.34	100	0	238
	5	1.33	1.26	0.82	0.65	100	0	345
	10	1.38	1.28	0.76	0.67	100	0	308
1 : 1 Cellulose/chitin mixture	1	0.17	0.18	0.39	0.09	48	52	119
	2.5	0.55	0.58	0.40	0.28	49	51	226
	5	1.36	1.24	0.42	0.65	51	49	329
	10	1.29	1.18	0.44	0.62	54	46	298

[a]Molar feed ratio of BTCA to the total anhydro-*N*-acetylglucosamine and anhydro-*N*-glucosamine residues in chitin, [b]Integrated fractions of resonance lines for CH, CH_2, and CH_3 in the solid-state ^{13}C spectra of chitin and cellulose/chitin hybrid hydrogels. The integrated fraction of the C1 line in each spectrum was set to 1, [c]Average number of BTCA molecules per monomer unit of hydrogel, [d]Molar percentages of chitin and cellulose in the hydrogels, [e]Water absorbency of each product after 72 h.

screened through a 16-mesh sieve to obtain a granular product. The chitin hydrogels listed in Table I were prepared according to a procedure similar to that described above by changing the molar feed ratio of BTCA. Cellulose hydrogels were also prepared from cellulose dissolved in LiCl/NMP using a method similar to the preparation chitin hydrogels for comparison.

Preparation of Cellulose/Chitin Hybrid Hydrogels

Cellulose/chitin hybrid hydrogels were prepared from a mixture of cellulose and chitin dissolved in LiCl/NMP using a method similar to the preparation of chitin hydrogels described above. Cellulose (0.43 g, 2.65 mmol for the monomer unit) was dissolved in an Erlenmeyer flask containing LiCl/NMP (2.5 g of LiCl and 47.5 g of NMP) under stirring at 500 rpm at room temperature for 2 days. To the cellulose solution was added a solution of chitin (0.50 g, 2.65 mmol) in LiCl/NMP (2.5 g of LiCl ad 47.5 g of NMP). After the mixture of cellulose and chitin solutions was well-mixed by stirring at room temperature for 2 h, 0.64 g of DMAP (5.3 mmol) was added to the mixture. After complete dissolution of DMAP, 1.1 g of BTCA (5.3 mmol), which corresponded to a 1 : 1 molar feed ratio of BTCA to the total monomers of cellulose and chitin, was added to the mixture. Esterification, precipitation for the hydrogels, neutralization of the unreacted BTCA, and purification were performed using methods similar to those described for the preparation of the chitin hydrogels. The cellulose/chitin hydrogels listed in Table I were prepared according to a procedure similar to that described above by changing the molar feed ratio of BTCA.

Structural Analysis

The Fourier transform infrared (FTIR) spectra of the hydrogels were measured using a PerkinElmer Spectrum Two spectrometer. FTIR spectra were obtained after grinding the sample into a powder and mixing with KBr powder. The powder mixture was compressed into a transparent disk and scanned from 4100 to 450 cm^{-1} using the average of 16 scans, with a resolution of 1 cm^{-1}. Dipolar decoupled solid-state ^{13}C NMR spectra were recorded on a Bruker Biospin Avance II 500 spectrometer (^1H frequency of 500 MHz) with a 4-mm dual-tuned MAS probe at a MAS frequency of 10 kHz. ^{13}C-excitation pulse with the flip angle of 30°, date acquisition time, and repetition time were set to 1.6 μs, 19 ms, and 20 s, respectively. During the data acquisition period, SPINAL-64 proton decoupling[22] was applied with a ^1H field strength of 100 kHz. The spectra were typically accumulated 6000–10,000 times to achieve a reasonable signal-to-noise ratio. D-Glycine was used as an external reference and ^{13}C chemical shifts were calibrated using the D-glycine carbonyl carbon resonance at 176.03 ppm. Line-shape analyses of the NMR spectra were performed using the Bruker TopSpin 3.0 software, and nonlinear least-squares methods were employed for line-fitting using previously described Gauss–Lorentz functions.[20,23,24]

Swelling Studies

The absorbency of the hydrogel products was determined according to the tea-bag method of the Japan Industrial Standard, JIS K7223.[20] A nylon tea bag with dimension of 100 × 200 mm was prepared from a 225-mesh nylon sheet using a heat sealer. The superabsorbent hydrogel sample (200 mg) was placed into the tea bag which was then immersed in water or the desired solutions at 25°C. After a prescribed time, the tea bag was removed from the aqueous solution and the excess water was drained for 10 min. The weight of the tea bag including the swollen hydrogels (W_h) was measured, and the water absorbency was calculated using the following equation:

$$\text{Water absorbency} = (W_h - W_b - W_d)/W_d,$$

where W_b is the weight of the empty tea bag after water treatment, and W_d is the weight of the dried superabsorbent hydrogel. As an external solution for determining the absorbency, distilled water and 50 m*M* citric acid–sodium citrate (pH 3), 50 mM NaH_2PO_4–Na_2HPO_4 (pH 7), and 50 m*M* glycine–NaOH (pH 10) buffer systems were used. Moreover, absorbency in aqueous 0.9 and 3.5 (w/v) % NaCl solution was investigated similarly. These concentrations are corresponding to those of physiological saline and seawater, respectively. These absorbency measurements

were taken for five samples of each hydrogel, and the average of the five values was plotted against the absorption time.

Biodegradability Test by Chitinase and Cellulase

Before performing the biodegradability test for the hydrogels, commercially available *Corynebacterium* sp. OZ-21 chitinase and *T. viride* ONOZUKA R-10 cellulase were purified at 4°C.[17] The following procedure for the purification of chitinase is provided as a representative example. Chitinase powder (10 g) was dissolved in 250 mL of 50 m*M* acetate buffer at pH 5.5. Ground-powdered $(NH_4)_2SO_4$ (180 g) was added to the solution to give 90% saturation. The precipitate that formed was collected by centrifugation, desalted by ultrafiltration using a Q0100 filter (Advantec, Japan), and then lyophilized. The cellulase powder was purified using the same procedure described for the purification of chitinase. The lyophilized chitinase and cellulase were used for the hydrogel biodegradability tests.

Chitinase activity was measured following the method of Jeuniaux et al.[25] using colloidal chitin as the substrate. The colloidal chitin was prepared from chitin using the method described by Sandhya et al.[26] To a mixture containing 9 mL of 0.2% (w/v) colloidal chitin in 50 m*M* sodium acetate buffer at pH 5.5 was added 10 mg of the purified chitinase dissolved in 1 mL of the same buffer solution. The mixture was incubated in a water bath at 40°C for 30 min. The reaction was arrested by addition of 3 mL dinitrosalicylic acid (DNS) reagent[27] followed by heating in a heat block at 100°C for 10 min. The colored solution was then centrifuged at 10,000 × *g* for 5 min and the UV absorbance of the supernatant was measured at 575 nm against the blank to determine the amount of reducing sugars in the mixture. Cellulase activity was measured using CMC as a substrate. A mixture of 9 mL of 1% (w/v) CMC in 50 m*M* sodium acetate buffer at pH 5.5 and 10 mg of the purified cellulase dissolved in 1 mL of the same buffer solution was incubated at 40°C. After 30 min, the amount of reducing sugars in the mixture was measured by the DNS method[27] described above. One unit of chitinase and cellulase activity was defined as the amount of enzyme liberating 1 μmol of reducing sugar per min. The protein masses of chitinase and cellulase were determined by use of the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as the standard.

The biodegradability test for the hydrogels was performed according to the method previously described for the determination of chitinase and cellulose activity.[20] A 100 mg sample of hydrogel was soaked in 18 mL of 50 m*M* acetate buffer, pH 5.5, for 2 days. Chitinase or cellulase dissolved in 2 mL of the same buffer solution was added to the suspension to give an activity of 0.20 U/mL and the mixture was incubated at 40°C. After a prescribed period, an aliquot (0.5 mL) was withdrawn from the mixture, and the amount of reducing sugars in the aliquot was determined by the DNS method described above. The biodegradability of the hydrogels was determined using the following equation:

$$\text{Biodegradability } (\%) = (M_s - M_b) \times 100/M_s,$$

where M_s is the molar mass of all residues in the hydrogel before degradation, and M_b is the molar mass of the reducing sugars liberated by the chitinase or cellulase.

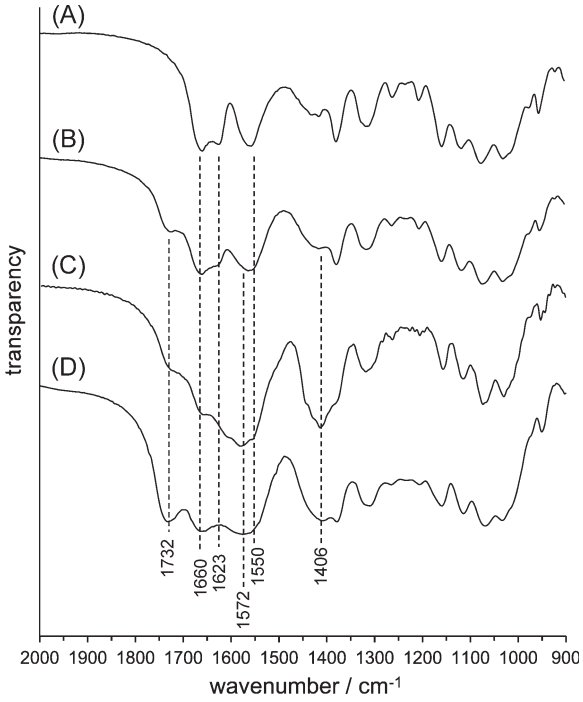

Figure 1. FTIR spectra of unmodified chitin (A) and a series of hydrogels obtained from the reaction of chitin with BTCA at the BTCA feed ratios of 1 (B), 2.5 (C), and 5 (D).

RESULTS AND DISCUSSION

Preparation and Structure of Chitin Hydrogels

Preparation of superabsorbent hydrogels from chitin was performed in the homogeneous reaction system of LiCl/NMP in the presence of DMAP as an esterification catalyst. Soon after adding the BTCA crosslinker, the reaction mixture became increasingly viscous, and the mixture changed from a liquid to a gel about 60 min after starting the reaction. Esterification was allowed to proceed with stirring at room temperature for 24 h, and then the reaction product was poured into the mixture of methanol and water. Subsequent conversion of the unreacted carboxyl groups to sodium carboxylates by the addition of aqueous NaOH was performed to enhance the water affinity of the product. Finally, the product was purified twice by reprecipitation with methanol and water, dried under reduced pressure, cut with a mixer, and then screened through a 16-mesh sieve to obtain a sandy-colored powder.

Figure 1 shows the FTIR spectra of the unmodified chitin and a series of products obtained by the esterification crosslinking of chitin with BTCA. As shown in Figure 1, the major peaks for chitin are at 1660 cm^{-1} for the C=O bonds of the acetamide groups (amide I band), and 1623 cm^{-1} and 1550 cm^{-1} for the N—H bending vibration of acetamide groups (amide II). 3328 After crosslinking esterification with BTCA, the spectra showed several new absorption bands in addition to the original peaks of pure chitin. The new absorption band at 1732 cm^{-1} was assigned to the C=O stretching vibration of the ester group, and those appearing at 1572 cm^{-1} and 1406 cm^{-1} were assigned to the asymmetric and symmetric stretching vibrations,

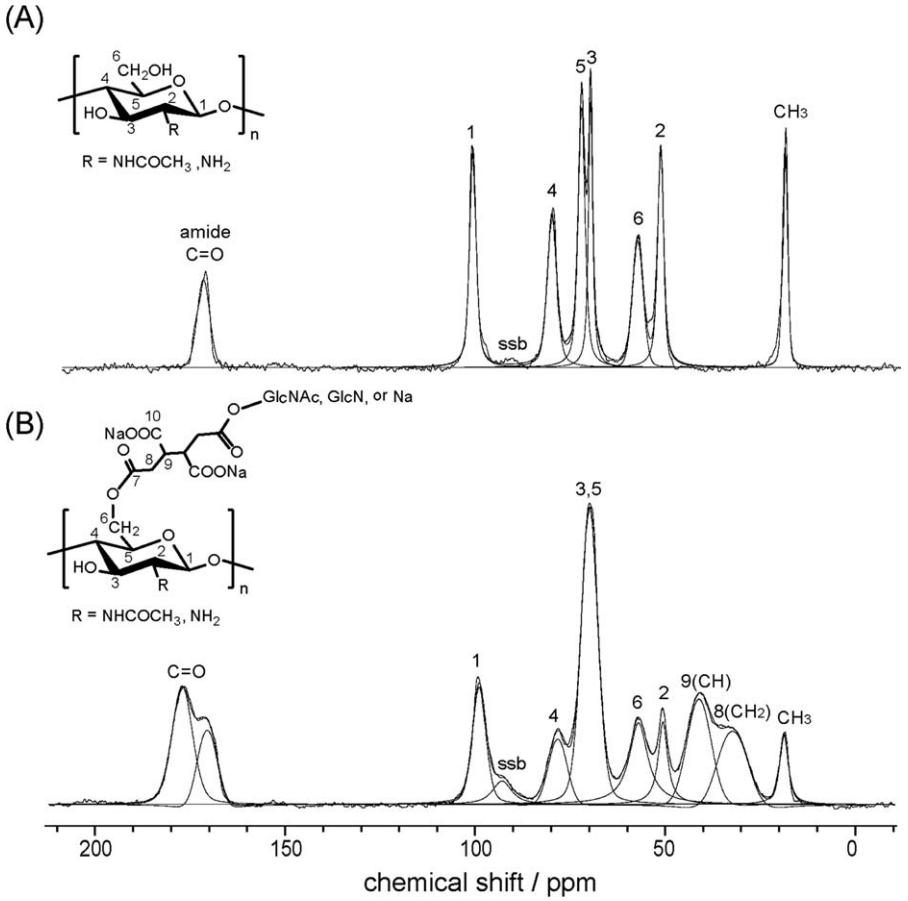

Figure 2. Solid-state ^{13}C NMR spectra of unmodified chitin (A) and the hydrogel product prepared from chitin with the BTCA feed ratio of 5 (B). Individual fit lines determined by the line-shape analysis are shown in this figure. The abbreviation "ssb" indicates spinning sidebands of the carbonyl carbon resonances.

respectively, of the C=O in the carboxylate group.[23,34] This clearly indicates that esterification between the hydroxyl groups of chitin and the acid anhydrides of BTCA occurred during the reaction in the LiCl/NMP system, resulting in the formation of carboxylate anions as well as crosslinking between the chitin chains. However, the formation of amide bonds between —NH_2 groups of GlcN in chitin and acid anhydrides of BTCA could not be clearly confirmed in these spectra.

Figure 2 shows the solid-state ^{13}C NMR spectra of pure chitin and chitin hydrogel prepared from BTCA crosslinking with chitin at the BTCA feed ratio of 5. The results of the nonlinear least-squares line-fitting analysis of the spectra are also shown in Figure 2. The spectra of chitin and the hydrogel were deconvoluted to 8 and 10 Lorentz–Gauss lines, not including the spinning side bands of the carbonyl carbons. When compared to the spectrum of chitin, three new resonance lines were observed in the spectrum of the reaction product. The lines at 46 and 33 ppm were assigned to the CH and CH_2 groups of the esterified BTCA, respectively, and the line at 178 ppm was assigned to the carbonyl carbons of the newly formed esters. As previously reported,[20] the molar ratio of sodium carboxylate groups to the ester linkages formed in the hydrogels prepared from cellulose with BTCA was represented as an area ratio of the two lines at 181 and 176 ppm. In the case of the chitin hydrogels, however, a detailed assignment of the carbonyl carbon region (186–164 ppm) could not be performed because the newly formed carbonyl carbons of the ester, carboxylate, and amide, and the carbons of the acetamide group of chitin were completely overlapped in the region.

Table I summarizes the integrated fractions of ^{13}C resonance lines for CH, CH_2, and CH_3 (I_{CH}, I_{CH2}, and I_{CH3}) of the hydrogel spectrum, where the integrated C1 line was set to 1. According to Scheme 1, when X mol of BTCA is esterified to 1 mol of the monomer unit of chitin, both CH and CH_2 carbons are increased by 2X mol. Thus, esterified BTCA molecules per one monomer unit of chitin (n_{BTCA}) were determined by eq. (1):

$$n_{BTCA} = (I_{CH} + I_{CH2})/4. \quad (1)$$

The n_{BTCA} values of a series of chitin hydrogels prepared using different BTCA feed ratios are also summarized in Table I. Based on the data summarized Table I, the n_{BTCA} values increased drastically as the feed ratio of BTCA increased up to 5. In addition, n_{BTCA} of the hydrogel prepared at the BTCA feed ratio of 10 was approximately the same as that prepared at the feed ratio of 5, suggesting that the crosslinking density was almost saturated at the feed ratio of 5.

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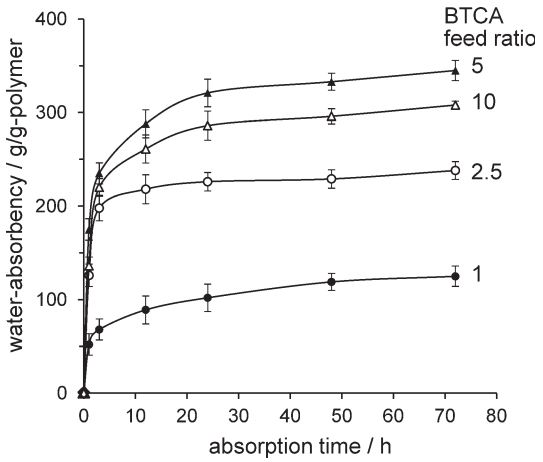

Figure 3. Time dependence of water absorbency of chitin hydrogels. The values in the figure indicate the ratio of BTCA molar feed to monomer unit of chitin (GlcN and GlcNAc). These absorbency measurements were taken for five samples of each hydrogel, and the average of the five values ($n = 5$) was plotted against the absorption time. The standard deviation value of each plot is shown in this figure.

Absorbency of Chitin Hydrogels

Sandy-colored granular products prepared from the crosslinking esterification reaction of chitin and BTCA absorbed water readily and formed transparent hydrogels upon soaking. Figure 3 shows the time dependence of the water absorbency of the hydrogels synthesized from chitin in which the ratio of BTCA feed to monomer unit of chitin was varied. The maximum absorbency of each sample was reached within about 48 h, and very little change in the absorbency was observed beyond 48 h. The saturated absorbency of each sample after 72 h is listed in Table I. Comparison of the hydrogel absorbencies shows that the absorbency increased with increasing BTCA feed ratio up to 5, and that the highest water absorbency of 345 g/g-polymer was obtained at the feed ratio of 5. This absorbency value is almost the same as that of SPA which is commonly used in practical applications. The absorbency of the sample prepared at the feed ratio of 10 was slightly lower than that prepared at the feed ratio of 5, indicating that the optimum n_{BTCA} was 0.65.

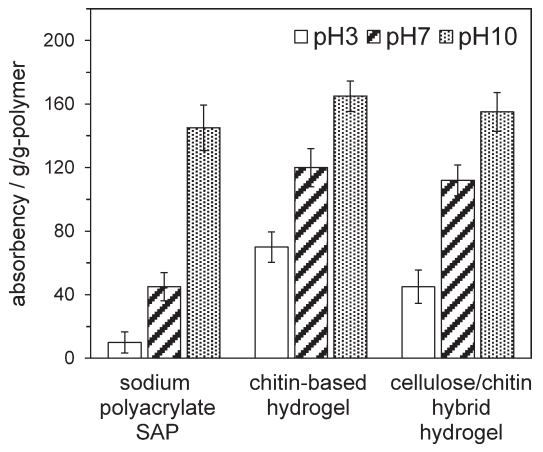

Figure 4. Comparison of Absorbency of SPA, chitin- and cellulose/chitin-based hydrogels in pH 3, 7, 10 buffer solutions after 72 h. The chitin- and cellulose/chitin hydrogels were prepared by the reactions at the BTCA feed ratio of 5. The standard deviation value of each absorbency ($n = 5$) is shown in this figure.

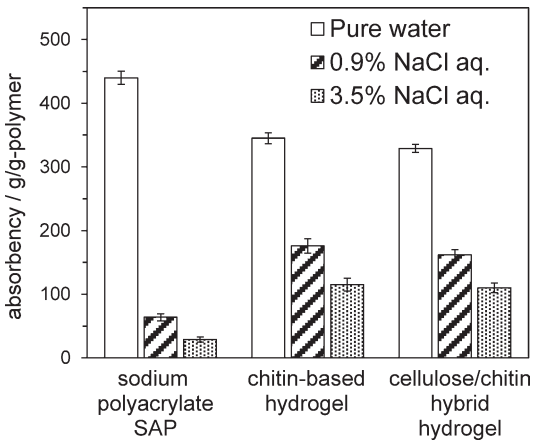

Figure 5. Comparison of Absorbency of SPA, chitin- and cellulose/chitin-based hydrogels in aqueous NaCl solutions after 72 h. The chitin- and cellulose/chitin hydrogels were prepared by the reactions at the BTCA feed ratio of 5. The standard deviation value of each absorbency ($n = 5$) is shown in this figure.

Figure 4 shows the absorbency of the chitin hydrogel prepared at the BTCA feed ratio of 5 and SPA after 3 days under different pH buffer conditions. Effect of pH on the swelling behavior of the chitin hydrogels is similar to that of the SPA although absorbency of the chitin hydrogel in each buffer solution differed appreciably from that of the SPA. Absorbency of these hydrogels increased markedly with the increasing buffer pH owing to the presence of carboxyl groups in both structures. In the neutral and alkaline pH region, because the dominant charged species in these hydrogels are unprotonated carboxyl group, the hydrogels are swollen owing to an intraionic repulsion between the unprotonated carboxyl groups.[29] In the case of acidic pH, on the other hand, the carboxyl groups are protonated, thereby lowering the ionic repulsion, which causes the gels to shrink.[29] The most remarkable characteristic of the chitin hydrogel was its absorbency in the acidic buffer solution of pH 3 (70 g/g), which was seven times as high as that of the SPA (10 g/g). The different swelling behavior of the chitin hydrogel in acidic pH is considered to be owing to the residual amino groups unreacted with BTCA in the structure. The residual amino groups are protonated in acidic pH, thus intraionic interaction between the positive charges is considered to prevent decrease of the swelling in the acidic solution.

Figure 5 shows the effect of NaCl concentration on the absorbency of the chitin hydrogel and SPA. In the case of SPA, osmotic pressure caused by the difference in mobile ion concentration between the gel inside and the solution was reduced in the solution with high NaCl concentration, thereby drastically lowering the absorbency of SAP.[29] On the other hand, the chitin hydrogels show the high absorbency in the aqueous NaCl solution, compared with SPA. The high absorbency of chitin hydrogel in the NaCl solution is considered to be owing to the

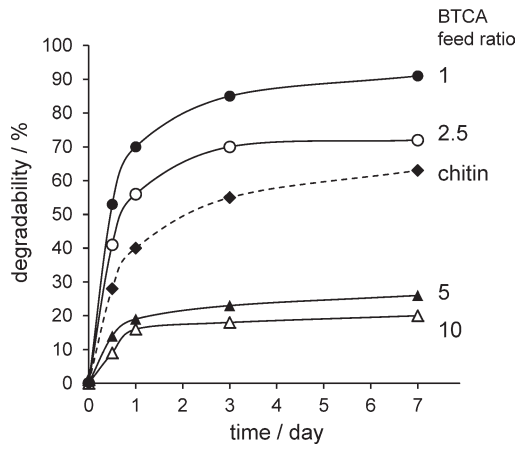

Figure 6. Chitinase degradation of chitin hydrogels. The dotted line indicates the degradation of unmodified chitin for comparison.

unreacted hydroxyl groups in the structure because the hydroxyl groups which are nonionic have a high affinity for water molecules.

Chitinase Degradation of Chitin Hydrogels

Enzymatic degradation of a series of chitin hydrogels was performed at 40°C in pH 5.5 buffer solution using *Corynebacterium* sp. OZ-21 chitinase. The time dependence of chitinase degradation for each hydrogel is shown in Figure 6. The dotted line in this figure represents the degradation profile of unmodified chitin for comparison. After 7 days of incubation with chitinase, 61% of the pure chitin degraded under the conditions employed. The hydrogels prepared from chitin also showed enzyme degradability, and degradation speed decreased with increasing BTCA feed ratio owing to the higher n_{BTCA} of the hydrogel structures. In particular, the products prepared at the BTCA feed ratios of 1 and 2 exhibited 91 and 72% degradability, respectively, which were much higher than the degradability of chitin. As the unmodified chitin has a highly crystalline hydrogen-bonded structure, only the fiber surfaces of chitin were degraded by chitinase. However, in the case of the hydrogels, the interchain distances expanded when the hydrogel is soaked in water, allowing chitinase to easily make contact with the interior chitin chains as well as the surface chains, resulting in high biodegradation. In the case of the chitin hydrogels prepared at the BTCA feed ratios of 5 and 10, degradability declined to 26 and 20%, respectively, because of the high n_{BTCA}.

Preparation and Structure of Cellulose/Chitin Hybrid Hydrogels

To prepare hydrogels which can be degraded by a wide variety of enzymes, hydrogels were prepared from a homogeneous mixture with a 1 : 1 molar ratio of cellulose and chitin using a procedure similar to that described for the preparation of the chitin hydrogels. As a result of the reaction, a milky white-colored powder was obtained. Like the chitin hydrogels, this powder readily absorbed water and changed to a transparent hydrogel upon soaking.

Figure 7 shows the FTIR spectra of a series of cellulose/chitin hybrid hydrogels prepared using different BTCA feed ratios along with comparison spectra for the hydrogels of chitin and cellulose. In the spectrum of the cellulose hydrogel, the bands at 1732, 1572, and 1406 cm^{-1} which were assigned to the C=O stretching vibration of the ester group, asymmetric C=O stretching vibration of the carboxylate in BTCA, and symmetric C=O stretching vibration of the carboxylate in BTCA, respectively.[20] In the case of the chitin hydrogel, the characteristic amide I band at 1660 cm^{-1} and the amide II band at 1550 cm^{-1} were observed.[28] In the spectrum of the cellulose/chitin hybrid hydrogel prepared at the BTCA feed ratio of 1, the bands at 1732, 1660, 1572, 1550, and 1406 cm^{-1} were observed, indicating that the cellulose and chitin were crosslinked by esterification with BTCA. The intensities of the ester band at 1732 cm^{-1} and carboxylate bands at 1572 and 1406 cm^{-1} increased as the BTCA feed ratio increased, indicating that the esterification of BTCA was enhanced by the increase of the BTCA feed ratio.

Figure 8 shows the solid-state ^{13}C NMR spectra of the hydrogels prepared from chitin, cellulose, and the 1 : 1 cellulose/chitin mixture. Line-shape analysis of the spectra provided the I_{CH}, I_{CH2}, and I_{CH3} for the cellulose/chitin hydrogel samples, and the n_{BTCA} value for each hybrid hydrogel was determined using eq. (1), as summarized in Table I. The n_{BTCA} values of the hybrid hydrogels increased as the feed ratio of BTCA increased, which is similar to the trend observed for the chitin hydrogels. Furthermore, the n_{BTCA} values for each hybrid hydrogel were almost the same as those of the corresponding chitin hydrogels, suggesting that the n_{BTCA} values were nearly independent of the ratio of cellulose and chitin. In addition, as the resonance line

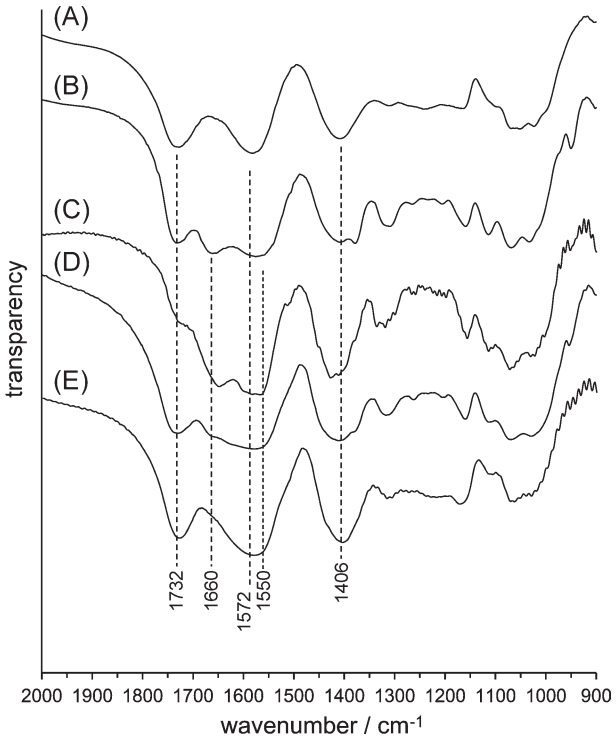

Figure 7. FTIR spectra of the chitin hydrogel with the BTCA feed ratio of 5 (A), cellulose hydrogel with the BTCA feed ration of 5 (B), and cellulose/chitin hybrid hydrogels. The cellulose/chitin hybrid hydrogels were prepared by reactions at the BTCA feed ratios of 1 (C), 2.5 (D), and 5 (E).

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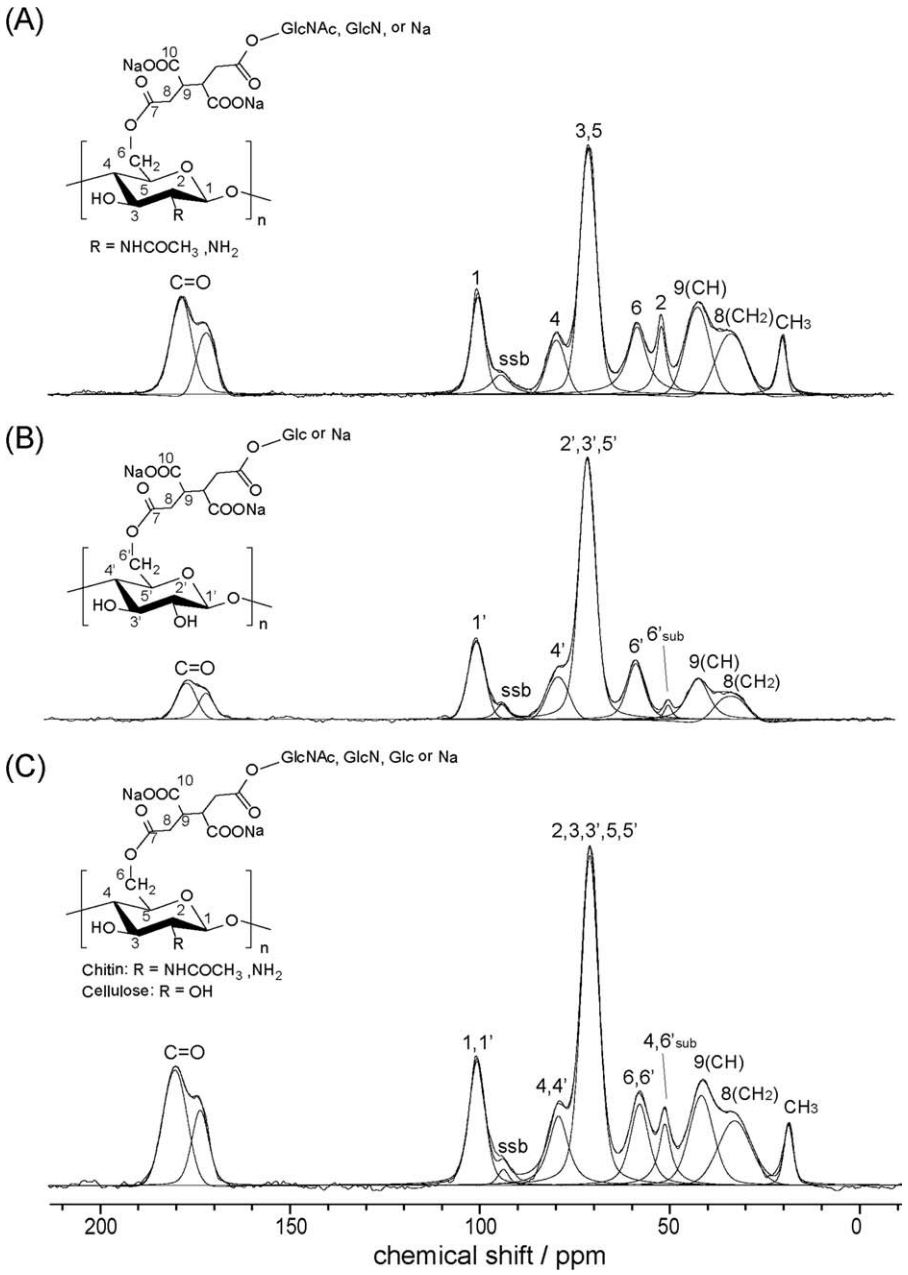

Figure 8. Solid-state ^{13}C NMR spectra of the chitin hydrogel (A), cellulose hydrogel (B), and cellulose/chitin hybrid hydrogels (C). These hydrogels were prepared at the BTCA feed ratio of 5. Individual fit lines determined by the line-shape analysis are shown in this figure. The abbreviations "ssb" and "sub" indicate spinning sidebands of the carbonyl carbon resonances and BTCA substituted, respectively.

of CH_3 was assigned to the acetamide group of chitin, the composition of the chitin and cellulose in the hydrogels could be determined from I_{CH3} using the following equations,

$$\text{Chitin composition} = (I_{CH3}/0.82) \times 100\ (\%),$$

and

$$\text{Cellulose composition} = (1 - I_{CH3}/0.82) \times 100\ (\%),$$

where the constant 0.82 was DA of the chitin. The chitin and cellulose composition for each hybrid sample is summarized in Table I. Chitin compositions of the hybrid hydrogels were 48–54%, indicating that the cellulose and chitin compositions of hybrid hydrogel were the same as the feed ratio of cellulose and chitin. In addition, the chitin and cellulose compositions of the hydrogel were independent of the feed ratio of BTCA.

Formation of the crosslinking between cellulose and chitin could not be clearly determined by the structural data from the FTIR and solid-state ^{13}C NMR. It was, however, revealed in the previous report that cellulose hydrogels could be obtained from wide variety of cellulose sources by the esterification crosslinking of BTCA in the same reaction condition.[20,24] In addition, as

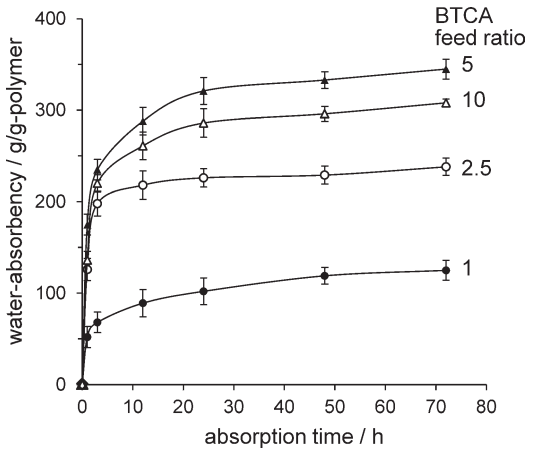

Figure 9. Time dependence of water absorbency of cellulose/chitin hybrid hydrogels. The values in the figure indicate the molar ratio of BTCA feed to polysaccharide monomer units (Glc, GlcN, and GlcNAc). The standard deviation value of each plot ($n = 5$) is shown in this figure.

cellulose and chitin were completely dissolved in the reaction solvent system employed in the study, it was considered that the formation of networks between cellulose–chitin, cellulose–cellulose, and chitin–chitin was occurred in the same proportion.

Absorbency of Cellulose/Chitin Hybrid Hydrogels

Figure 9 shows the time dependence of the water absorbency of hybrid hydrogels in which the ratios of BTCA feed to monomer unit of cellulose/chitin were varied, and the saturated absorbency of each sample after 72 h is summarized in Table I. Comparison of the absorbencies of the hybrid hydrogels at various BTCA feed ratios shows that the highest water absorbency of 329 g/g-polymer was obtained at the BTCA feed ratio of 5. The value of n_{BTCA} for the product exhibiting maximum water absorbency was 0.65, which is in agreement with the n_{BTCA} value of the most absorbent chitin hydrogel. In addition, the n_{BTCA} values and the maximum water absorbencies of the other hybrid hydrogels were approximately the same as those of the corresponding chitin hydrogels, suggesting that incorporation of cellulose to chitin hydrogel has no strong influence on the water absorbency.

On the other hand, incorporation of cellulose to chitin hydrogels slightly affected to swelling behavior in different pH solutions (Figure 4). The absorbency of the hybrid hydrogels obtained at the BTCA feed ratio of 5 in buffer solution of pH 3 was much lower than that of the corresponding chitin hydrogel. As the chitin composition of the hybrid hydrogel was about 50%, the ionic repulsion caused by protonated amino groups in the hybrid hydrogels in the acidic solution was considerably lower than that in the chitin hydrogel. Therefore, it was considered that the chitin composition was important factor to absorb acidic solution in applications. As shown in Figure 5, absorbencies of the hybrid hydrogel in aqueous 0.9 and 3.5% NaCl solutions were almost the same as those of the corresponding chitin hydrogel. As hydroxyl groups in cellulose unreacted with BTCA has an affinity with water like hydroxyl groups of chitin, incorporation of cellulose to chitin hydrogel has no strong influence on the water absorbency in aqueous NaCl solutions.

Chitinase and Cellulase Degradation of Cellulose/Chitin Hybrid Hydrogels

Enzymatic degradation of the hybrid hydrogels was performed using chitinase and cellulase. The time dependence of degradation by chitinase is shown in Figure 10(A). The dotted lines in the figure represent the chitinase degradation of unmodified cellulose and chitin. After 7 days of degradation by chitinase, 63% of chitin and 31% of cellulose were degraded under the conditions employed. This indicates that the chitinase used in this study has cellulase activity. The hybrid hydrogels were also biodegradable in the presence of chitinase, and the hydrogels prepared at the BTCA feed ratios of 1 and 2.5 exhibited degradation of 81 and 54%, respectively. The degradation speed decreased with increasing BTCA feed ratio owing to the higher n_{BTCA} of the structure. Figure 10(B) shows the time dependence of the degradation by cellulase. In this case, 62% of cellulose was degraded, whereas the chitin was not degraded, which indicates that the cellulase has no chitinase activity. The hybrid hydrogels were also degraded by cellulase, and the degradation speed decreased with increasing BTCA feed ratio. In particular, the hybrid hydrogel obtained at the BTCA feed ratio of 1 was 52% cellulose and exhibited 44% degradability, whereas the hydrogel obtained at BTCA feed rate 2.5 was 51% cellulose and exhibited 37% degradability. This indicated that the cellulose components of the hybrid hydrogels obtained at the BTCA feed ratios of 1 and 2.5 were degraded by 88 and 74%, respectively.

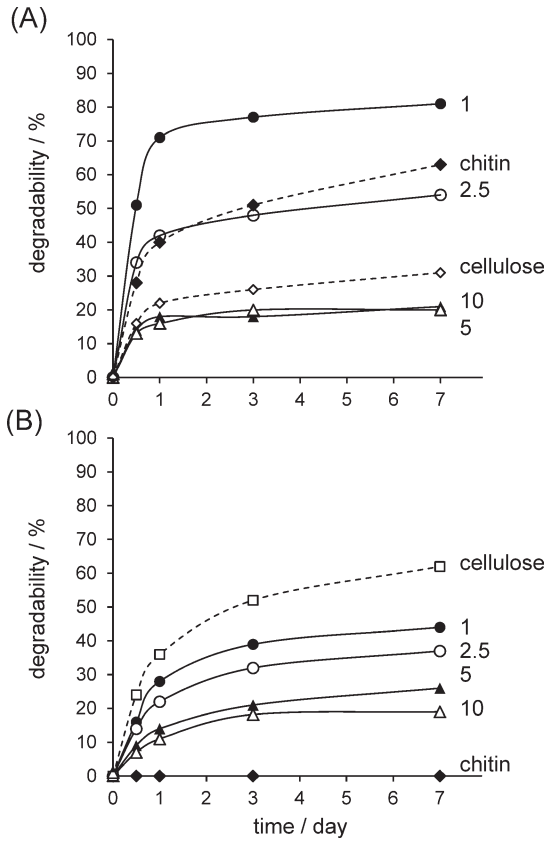

Figure 10. Chitinase (A) and cellulase (B) degradation of cellulose/chitin hybrid hydrogels. The dotted lines indicate the degradation of unmodified chitin and cellulose powder for comparison.

Therefore, the hybrid hydrogels have the benefit of the degradability by both cellulase and chitinase.

CONCLUSIONS

Novel biodegradable superabsorbent hydrogels were prepared from chitin dissolved in LiCl/NMP via simple esterification crosslinking with BTCA under mild conditions. The ratio of BTCA feed to chitin strongly influenced n_{BTCA}, and the feed ratio of 5 provided the hydrogel with maximum water absorbency. In addition, cellulose/chitin hybrid hydrogels were also obtained from the homogeneous mixture of cellulose and chitin by a method similar to that for the preparation of the chitin hydrogels. The percentages of cellulose and chitin in the hydrogels were reflected by the molar feed ratios of cellulose and chitin, and the BTCA feed ratio to cellulose/chitin also influenced n_{BTCA} and water absorbency.

When comparing SPA, the chitin and cellulose/chitin hybrid hydrogels showed good absorbency in acidic pH as well as aqueous NaCl solution owing to the amino and hydroxyl groups unreacted with BTCA. Drastic decrease of water absorption ability in acidic and high salt concentration solution has been remained as one of the drawbacks of SPA in spite of wide spread use of this polymer.[1] The improvement of the absorbency in acidic solution and NaCl solution by use of the chitin and cellulose/chitin hydrogels should be emphasized because SAP should absorb acidic and aqueous NaCl solutions in many applications such as sand-bag, water-holding agent for soil, gelling agent for cell electrolytes and so on.

These chitin and cellulose/chitin hybrid hydrogels exhibited good degradability by chitinase, and the hybrid hydrogels were also degraded by cellulase. Therefore, the chitin or cellulose/chitin hydrogels are expected to be applicable for agricultural and industrial uses and should be viable alternatives to SPA.

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