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SYNTHESIS AND CHARACTERIZATION OF POLY(ASPARTIC ACID) AND ITS DERIVATIVES AS BIODEGRADABLE MATERIALS

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Key Words: L-Aspartic Acid, Poly(aspartic acid), Poly(succinimide)

ABSTRACT

Three types of modified poly(aspartic acid)s, such as poly(aspartic acid-co-aminocarboxylic acid) (**4**), alkylamine modified poly(aspartic acid) (**5**) and crosslinked poly(aspartic acid) (**6**), were synthesized and calcium-ion chelating ability, hygroscopicity and water absorption were evaluated. The calcium-ion chelating ability of **4** depended on the kind of aminocarboxylic acids and the content of aminocarboxylic acid in the copolymer. The highest value was 3 times higher than that of poly(acrylic acid) with a M_w of 14000. The highly modified PASP, e.g., 50 mol% lauryl amine modified poly(aspartic acid), showed the highest hygrogroscopicity among homopoly(aspartic acid)s and mod-

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ified poly(aspartic acid)s. The maximum swelling of poly(aspartic acid) hydrogel prepared by the γ-irradiation of homopoly(as-partic acid) was 3400 g-deionized water/g-dry hydrogel.

INTRODUCTION

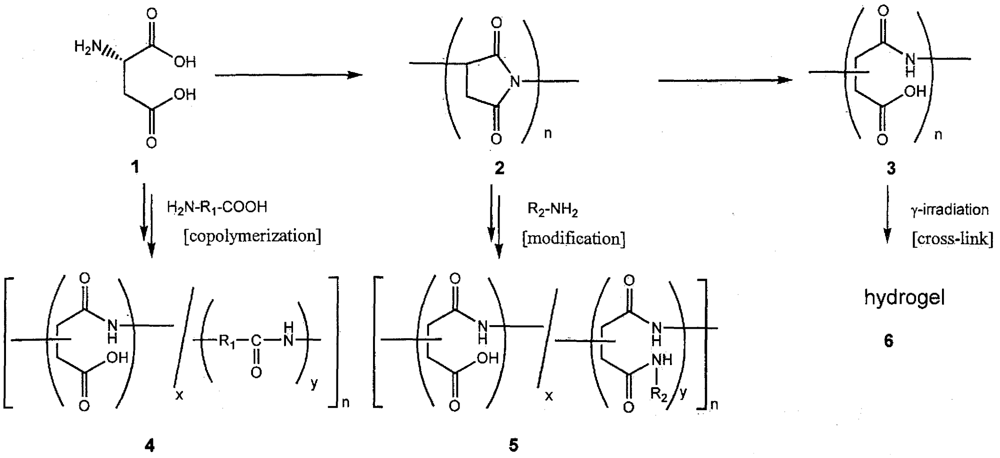
Water-soluble polymers, such as poly(vinyl alcohol), poly(ethylene glycol) and poly(acrylic acid), are widely used as cosmetics, paper additives, dispersants, and detergent builders, but they are rarely recovered or collected after use. Of concern, is the diffusion and accumulation of such nonbiodegradable water-soluble polymers in the earth's environment after its release. Polymers with carboxylic acid groups are one of the most important water-soluble polymers, e.g., poly(acrylic acid) and poly(methacrylic acid) have been used as detergent builders, scale inhibitors and flocculants and are directly released into the earth's environment. However, they are hardly biodegradable except for their oligomers [1-3], which will possibly produce much damage to the environment. Therefore, the biodegradable substitutes for nonbiodegradable polymers having carboxylic acid groups are really desired in terms of the earth's environment.

Poly(amino acids) having free carboxylic-acid groups, such as poly(aspartic acid) (PASP) and poly(glutamic acid), are candidates for the biodegradable water-soluble polymer. The thermal polycondensation of aspartic acid is known to easily produce PASP, so that it has a great potential to resolve environmental problems, in particular, in the field of biodegradable water-soluble polymer [4].

There is much research about the production of polysuccinimide (PSI) that can be hydrolyzed to PASP [5-8], but they have many disadvantages, including low molecular weight, difficulty of isolation and low biodegradability.

Recently, we reported the synthesis of a high-molecular-weight PSI prepared by the polycondensation of L-aspartic acid using acid catalyst in various solvents [9]. However, the calcium-ion chelating ability was insufficient to use PASP in place of poly(acrylic acid) and poly(acrylic acid-*co*-maleic acid). In addition, we reported the synthesis of poly(succinimide-*co*-6-aminocaproic acid) with various feed ratios and its hydrolyzed copolymer [10].

In this study, we report the synthesis of three types of modified poly(aspartic acid)s which are copoly(aspartic acid), alkylamine modified PASP and crosslinked PASP (Scheme 1), and the properties of them which are focused on calcium-ion chelating ability, hygroscopicity and water absorption.



Scheme 1. Modifications of poly(aspartic acid).

EXPERIMENTAL

Materials and Measurements

L-Aspartic acid (ASP) was obtained from Mitsubishi Chemical Corporation (Japan). 85%-*o*-phosphoric acid, aminocarboxylic acid, mesitylene, sulfolane, *N,N*-dimethylformamide (DMF), sodium hydroxide and deionized water were commercially available and used without further purification. ^{1}H-NMR spectra were measured with JEOL JNM-GSX400 and Bruker ASX300 spectrometers. The molecular weight of PSI was estimated in DMF containing 20 mmol/L of LiBr by gel permeation chromatography (GPC) (column: PLgel 5 mm MIXED-C x 2, detector: refractive index, standard: polystyrene). The residual amount of ASP after the polycondensation was measured in water containing 2.5 g/L of H_3PO_4 and 31.2g/L of $NaH_2PO_4 \cdot 2H_2O$ by liquid chromatography (LC) (column: Shim-pack ISC-07/S1504, detector: UV 210 nm).

Preparation of Poly(aspartic acid)

PASPs of several molecular weights were synthesized by the polycondensations reported in previous papers [5-9] with or without solvent and acid catalyst, and then alkali hydrolysis. A typical procedure for the polycondensation with solvent and acid catalyst is as follows: A suspension of ASP (25 g, 0.188 mol) and 85%-*o*-phosphoric acid (9.4 mmol) in mesitylene (56 g) and sulfolane (24 g) was refluxed under a N_2 atmosphere. Water formed in a reaction mixture was removed using a Dean-Stark trap with a reflux condenser. After 4.5 hours, the solvent was removed, then the precipitate was washed with water (200 mL) several times until it was neutral. The residue was washed with MeOH (200 mL) and dried at 85°C under reduced pressure to yield PSI (17.5 g, 96%). To a 100 mL beaker with a stirring bar, PSI (3 g) and a solution of sodium hydroxide (1.4 g, equivalents per succinimide residue), and deionized water (20 mL) under ice cooling were added. After the mixture was stirred for 1 hour, the reaction mixture was poured into methanol (300 mL), and then the precipitate was filtered and dried at 40°C under reduced pressure to yield PASP sodium salt (3.9 g, 95%).

Acid-Catalyzed Copolycondensation of ASP and Aminocarboxylic Acid

a) Preparation of Poly(succinimide-co-aminocarboxylic acid)

A typical procedure for the polycondensation of ASP with 6-aminocaproic acid is as follows: ASP (2.5 g, 19 mmol), 6-aminocaproic acid (1.1 g, 8.1 mmol), and 85% phosphoric acid (2.5 mg, 2.2 mmol) in mesitylene/sulfolane (16 mL/5 mL) were placed in a 100 mL flask equipped with a Dean-Stark trap. The reaction mixture was refluxed with stirring at 180°C for 4.5 hours. Mesit-ylene was evaporated from the reaction mixture under reduced pressure. The residual crude product was purified by reprecipitation with DMSO-MeOH and then the precipitate was filtered off and dried in vacuum to yield the MeOH-in-soluble polymer (2.3 g, 84% yield).

b) Alkali Hydrolysis of Poly(succinimide-co-aminocarboxylic acid)

To a 0.1 N NaOH aqueous solution (10 mL) was added poly(succinimide-*co*-6-aminocaproic acid) (100 mg) with stirring in an ice-bath for 20 minutes, and then the polymer was dissolved in the solution. This polymer solution was dialyzed against water for 24 hours and then lyophilized.

Preparation of Alkylamine Modified Poly(aspartic acid)

Alkylamine modified PASP was prepared by the aminolysis of PSI using lauryl amine and DMF as a solvent, and the hydrolysis with aqueous NaOH solution. A typical procedure for the preparation of a lauryl amine modified PASP is as follows: PSI (5.0 g) and DMF dried with MS4A were placed in a 100 mL flask equipped with a reflux condenser under N_2. Lauryl amine (1.0 g, 5.1 mmol) was added to the mixture, and then the reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was poured into water (100 mL). After filtration, the precipitate was washed with water (100 mL) and MeOH (100 mL), and dried at 100°C for 24 hours under reduced pressure to yield an amine modified PSI (5.25 g, 89%). To a 50 mL beaker with a stirring bar and a solution of NaOH (1.0 g, 25 mmol) and deionized water (37.5 mL), an amine modified PSI (2.5 g) was added under ice cooling. After the mixture was stirred for 24 hours, the reaction mixture was poured into methanol (300 mL), and then the precipitate was filtered and dried at 50°C under reduced pressure to yield a modified PASP sodium salt (3.0 g, 89%).

Preparation of Poly(aspartic acid) Hydrogels

PASP hydrogels were prepared by γ-irradiation (1.6 kGy/h) of PASP aqueous solutions (1-25 wt/vol%) using an irradiation system with a ^{60}Co (110 TBq) source [11]. The PASP solution (2 mL) was contained in a 10-mL glass vial capped under nitrogen and air. The resultant hydrogels were swollen to equilibrium for 1 week at room temperature. During this time, the uncrosslinked PASP was removed by changing the swelling media daily.

The ratio of gelation was calculated as

$$\text{ratio of gelation (\%)} = (W_l / W_f) \times 100$$

where W_f is the weight of PASP feed in the vial and W_l is the dry weight of the PASP hydrogel after the removal of the uncrosslinked PASP.

Calcium-Ion Chelation by Poly(aspartic acid)

The calcium-ion chelating ability of the various polymers was determined using a calcium-ion electrode and an ion meter in accordance with the description in a previous paper [12]. A sample (10 mg) was dissolved in 50 mL of an aqueous solution, which had been adjusted to give a calcium chloride concentration of 1.0×10^{-3} mol/L and a potassium chloride concentration of 0.08 mol/L. The resulting mixture was stirred at 30°C for 10 minutes and the calcium ions in the solution were determined using a calcium-ion electrode (Orion Model 93-20) and an ion meter (Orion Model 720A).

Hygroscopicity of Poly(aspartic acid)

The hygroscopicity of PASP was measured using a constant temperature and constant moistened bath (Seiwa Rikou Co. Ltd., Japan). The hygroscopic cycle was used the description in the previous paper [13]. The sample dried under reduced pressure was placed for 7 days in the bath which was controlled

65% R.H. at 25°C and then for 7 days in the bath which was controlled 35% R.H. at 25°C. The hygroscopicity and the ratio of hygroscopicity was calculated as

$$\text{hygroscopicity at 35\% R.H. (\%)} = (W_{14}-W_0) \times 100/W_0$$

$$\text{ratio of hygroscopicity (\%)} = W_7 \times 100/W_{14}$$

where W_n is weight of the sample after n days.

Biodegradability of Poly(aspartic acid)

The biodegradability of PASP was estimated using OECD 301C (Modified MITI Test). A sample was treated with the standard activated sludge, which was obtained from the Chemicals Inspection & Testing Institute, Japan, at 25 ± 1°C for 28 days. Aniline was used as the standard to check the activity of the standard activated sludge. Biological oxygen demand (BOD) and total organic carbon amount (TOC) are the consumption of the oxygen and total organic carbon amount during the evaluation, respectively. Both are generally used for evaluating biodegradability. BOD and TOC were measured using an OM3001 coulometer (Ohkura Electric Co., Ltd., Japan) and a Total Carbon Analyzer TOC-5000A (Shimadzu Corporation, Japan), respectively. Removed TOC was calculated from the difference between the amount of the total organic carbon before and after the evaluation of biodegradability.

RESULTS AND DISCUSSION

Synthesis of Copoly(aspartic acid)

The copolycondensation of ASP and various aminocarboxylic acids with *o*-phosphoric acid as a catalyst was carried out under reflux in mesitylene/sulfolane (Table 1). Poly(succinimide-*co*-aminocarboxylic acid)s were obtained by the reactions in high yield. In these polycondensations, all GPC charts showed unimodal peaks, indicating that no homopolymer of ASP or the aminocarboxylic acids was produced. For aliphatic linear aminocarboxylic acids, polymer yields decreased with increasing methylene number and higher molecular weights, ca. 21000, were obtained for the methylene number of 2 and 11, 3-aminopropionic acid and 12-aminolauric acid. For the aromatic aminocarboxylic acids, the obtained polymers were soluble in DMF for *p*-aminobenzoic acid and 4-aminophenylacetic acid, while they were insoluble for *p*-aminomethylbenzoic

TABLE 1. Polycondensation of L-Aspartic Acid and Various Aminocarboxylic Acid with *o*-Phosphoric Acid[a]

Comonomer	Yield (%)	Mole Fraction of Succinimide Unit in Copolymer[b)]	Mw(Mw/Mn)[c)]
3-aminopropionic acid	94	70	6230(1.75)
4-aminobuthylic acid	92	88	21300(2.12)
6-aminocaproic acid	84	76	14000(1.64)
11-aminoundecanoic acid	80	79	14400(1.70)
12-aminolauric acid	79	71	21200(1.41)
D,L-α-amino-n-caprylic acid	94	85	7230(2.10)
4-aminocyclohexane carboxylic acid	82	84	6300(1.67)
1-aminocyclohexane carboxylic acid	61	91	13800(1.65)
p-aminobenzoic acid	65	80	3600(1.26)
p-aminophenylacetic acid	98	84	11000(1.26)
p-aminomethylbenzoic acid	100	n.d[d)]	n.d[e)]
p-aminocinnnamic acid	100	n.d[d)]	n.d[e)]

[a)] ASP/comonomer feed mole ratio, 7/3; solvent, mesitylene/sulfolane (7/3 wt ratio); *o*-phosphoric acid, 8 mol% of ASP+amino carboxylic acid; temp, 180 °C; time, 4.5h.

[b)] Determined by ^{1}H,^{13}C-NMR spectra.

[c)] Determined by GPC in DMF using polystyrene as a standard.

[d)] The mole fraction of succinimide unit in copolymer could not measure, because the polymer was insoluble in NMR solvent.

[e)] The molecular weight could not measure, because the polymer was insoluble in DMF.

acid and *p*-aminocinnamic acid. The compositions of succinimide units in all copolymers, which were determined from ^{1}H-NMR spectra, tends to be higher than those of ASP in the monomer feed, indicating that the reactivity of ASP is higher than those of the aminocarboxylic acids during the acid-catalyzed polycondensation. In order to obtain copoly(aspartic acid)s, the hydrolysis of poly(imide-amide) was carried out using 0.1N NaOH aqueous solution. The ratio of α- and β-openings, which was determined by the area ratio of ^{1}H NMR spectrum, was estimated to be ca. 1/4, which indicated that the β-opening predominantly occurred rather than the α-opening. The ratio of α-opening/β-opening is similar to that of PASP. This means that the comonomer unit does not affect the hydrolysis of succinimide unit in the copolymer.

Calcium-Ion Chelating Ability of Poly(aspartic acid)

The chelating ability toward calcium ion of PASP is about a half of that of poly(acrylic acid) (Figure 1). The copolymers consisting of ASP and aliphatic and linear aminocarboxylic acids produced to improve chelating abilities toward calcium ion. The chelating abilities depended on the kind of aminocarboxylic acids and the content of aminocarboxylic acid in the copolymer as shown in Figures 2 and 3, respectively. Both results indicate that the chelating ability and the effectiveness of the carboxylic acid in PASP for calcium ion remarkably

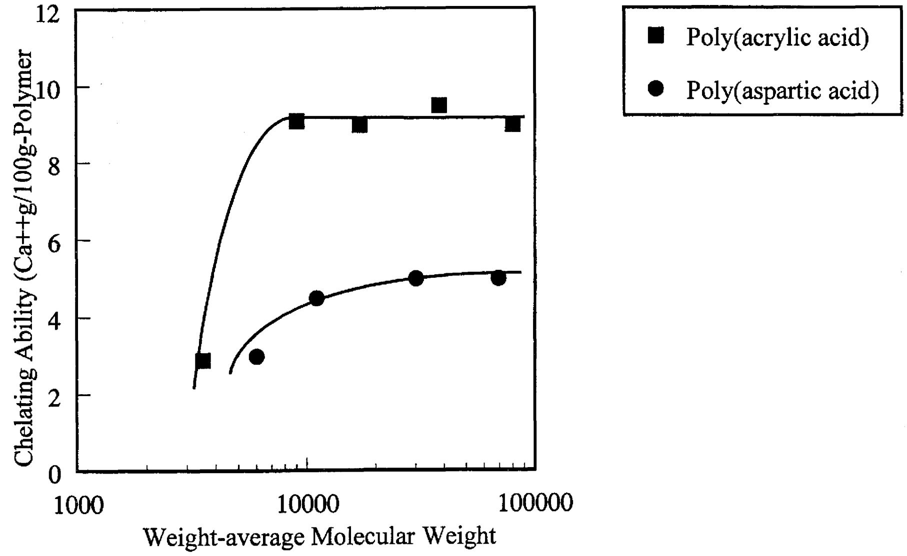


Figure 1. Chelating abilities of homopolymer.

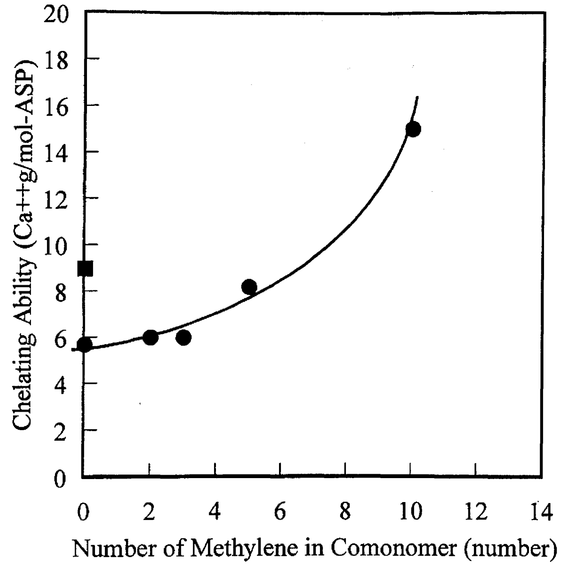


Figure 2. Chelating abilities of poly(aspartic acid-co-aminocarboxylic acid).

increase with increasing hydrophobic character of the copolymers. The polymer conformation in water should change by introducing a hydrophobic segment into the polymer, which affects the chelating ability of PASP. The highest value was 3 times higher than that of poly(acrylic acid) with a M_w of 14000.

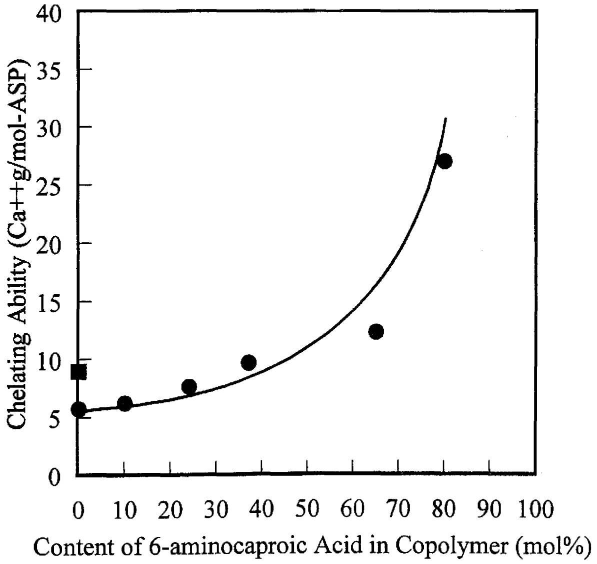


Figure 3. Chelating abilities of copolymer of ASP with 6-aminocaproic acid.

Hygroscopicity of Poly(aspartic acid)

The hygroscopicity of poly(aspartic acid) produced by various procedures and several references, for example L-lactic acid sodium salt and hyaluronic acid sodium salt was measured in various temperatures and humidities. The hygroscopicity of PASPs at 25°C were lower than that of hyaluronic acid sodium salt and higher than that of L-lactic acid sodium salt. PASPs showed the very similar features and the effect of the difference of the procedures and the structures was not detected (Figure 4). In the case of modified PASP, although the hygoscopicities of a 10 mol% laurylamine modified PASP and poly(aspartic acid-*co*-6-aminocaproic acid) (molar ratio 70/30) showed similar to that of PASP, a highly modified PASP, e.g., 50 mol% lauryl amine modified PASP, showed a different character (Figure 5). This result shows that the hygrosopicity is controlled by the amount of alkyl group. Therefore, the balance of hydro-philic and hydrophobic unit in the polymer should act an important role on the hygrosopicity.

Hydrogel of Poly(aspartic acid)

PASP hydrogels have been prepared by the γ-irradiation of PASP produced by thermal polycondensation reactions with an acid catalyst. [11] The effects of the molecular weight of PASP, pH, concentration of PASP in the aqueous solution and dosage of γ-irradiation on the PASP hydrogel preparation were

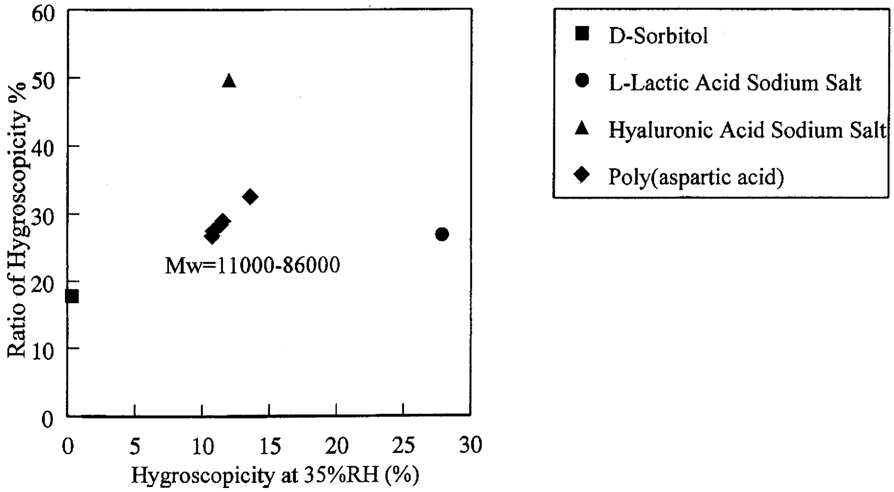


Figure 4. Hygroscopicity of poly(aspartic acid).

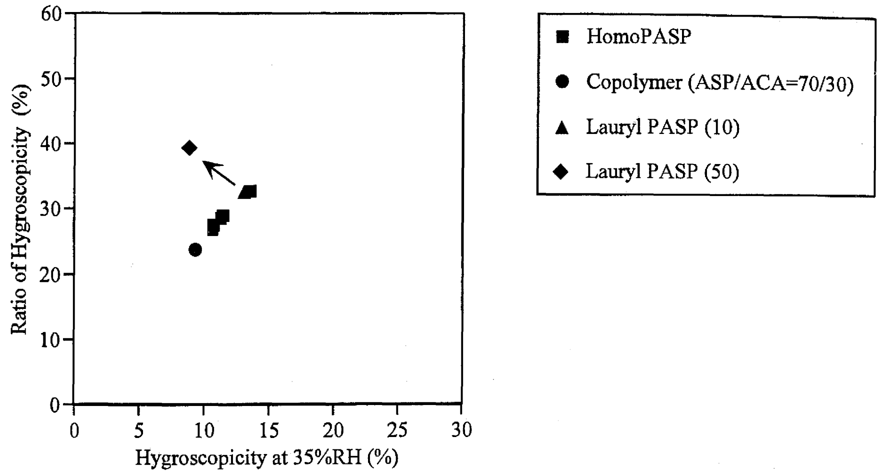


Figure 5. Hygroscopicity of modified poly(aspartic acid).

investigated. PASP hydrogels were prepared in the case when the PASP of M_w was 95000, the pH of the solution was 7.5 or higher, the concentration of the solution was 5-10 wt/vol%, the dosage of γ-irradiation was 32 kGy or more and the preparation of PASP solutions was done under N_2 (Table 2). On the other hand, PASP with low-molecular weight (M_w = 15000) could not formed hydrogel by γ-irradiation. The swelling of PASP hydrogels in deionized water and artificial urine was measured. The maximum swelling by deionized water was 3400 g-water/g-dry hydrogel.

TABLE 2. Specific Water Content of Poly(aspartic acid) Hydrogels

PASP Conc. (wt/vol%)	γ-Irradiation Dosage (kGy	Specific Water Content (g/g) pH = 3	pH = 7.5	pH = 13
5	32	NG[b)]	NG	1000
5	56	NG	NG	3200
5	63	NG	1100	3100
5	100	NG	820	2500
10	55	NG	NG	NG
10	63	NG	3400	NG

a) Specific water content is (weight of absorbed water / weight of dry hydrogel).

b) NG is no gel formation.

Biodegradability of Poly(aspartic acid) and its Derivatives

The biodegradation of PASP and modified PASPs was measured using activated sludge according to the method of OECD 301C and biodegradability was evaluated by the value of total organic carbon (TOC). Table 3 lists the TOC values for poly(aspartic acid) and modified PASPs. All modified PASPs except the cross-linked PASP showed the lower biodegradability than that of PASP. Furthermore, there is a marked decline in the modification of PASP with aminolysis, such as 10 mol% lauryl amine modification, as compared with copolymerization of ASP and 6-aminocaproic acid (molar ratio 90/10). These results show that the hygrophobic character in the modified PASP decreases the biodegradability. It is presumed that the association between a certain enzyme in activated sludge and the modified PASP may be affected by the modification.

CONCLUSION

Three types of modification of poly(aspartic acid) were conducted to improve the performance of homopoly(aspartic acid) in several applications. The acid-catalyzed copolycondensation of L-aspartic acid and aminocarboxylic acid was carried out using mesitylene/sulfolane solvent system and produced copolymer in high yield. The calcium-ion chelating ability, hygroscopicity and the performance of hydrogel produced by the γ-irradiation of poly(aspartic acid)

TABLE 3. Biodegradability of Poly(aspartic acid) and Modified Poly(aspartic acid)s

Sample	Mole Fraction of Succinimide Unit in Copolymer	Biodegradability[a] (TOC/%)
poly(aspartic acid)[b]	100	82.0
poly(aspartic acid-*co*-3-aminopropionic acid)[c]	70	3.5
poly(aspartic acid-*co*-4-aminobuthylic acid)[c]	80	16.3
poly(aspartic acid-*co*-6-aminocaproic acid)[b]	90	78.2
poly(aspartic acid-*co*-6-aminocaproic acid)[b]	76	61.6
poly(aspartic acid-*co*-6-aminocaproic acid)[b]	63	27.7
poly(aspartic acid-*co*-11-aminoundecanoic acid)[c]	79	13.6
10mol% lauryl amine modified poly(aspartic acid)[d]	-	20.5
poly(aspartic acid) hydrogel[e]	-	44.5

a) Using activated sludge; period, 28 days; temp, 25± 1°C.
b) Solvent, Mesityle/sulfolane; catalyst, H_3PO_4; temp, 180 °C; time, 4.5h.. See reference [9, 10].
c) ASP/comonomer feed mole ratio, 7/3; solvent, Mesityle/sulfolane; catalyst, H_3PO_4; temp, 180 °C; time, 4.5h.
d) See experimental section.
e) The weight-average molecular weight of PASP before γ-irradiation was 95000. The condition prepared PASP hydrogel was 100kGy γ-irradiation dose, 5 wt/vol% PASP concentration in the solution and pH 7.5 of the solution.

were measured. The chelating ability remarkably increased by the copolymerization with aminocarboxylic acid. The highest value was 3 times higher than that of poly(acrylic acid). The hygroscopicity is also increased by the introduction of alkyl aspartamide structure into the polymer. The hydrogel was produced by the γ-irradiation of poly(aspartic acid) and it showed that the maximum swelling by deionized water was 3400 g-water/g-dry hydrogel.

REFERENCES

[1] T. Hayashi, H. Nishimura, K. Sakano, and Y. Tani, *Biosci. Biotech. Biochem.*, *58*, 444 (1994).
[2] T. Hayashi, M. Mukouyama, K. Sakano, and Y. Tani, *Appl. Environ. Microbio.*, May, 1555 (1993).
[3] T. Hayashi, *Bioscience & Industry*, *53*, 521 (1995).
[4] J. Kovacs, I. Koenyves, and A. Pusztai, *Experientia*, *9*, 259 (1953).
[5] A. Vegotosky, K. Harada, and S. W. Fox, *J. Am. Chem. Soc.*, *80*, 3361 (1958).
[6] K. Harada, *J. Org. Chem.*, *24*, 1662 (1959).
[7] J. Kovacs, H. N. Kovacs, I. Konyves, J. Csaszar, T. Vajda, and H. Mix, *J. Org. Chem.*, *26*, 1084 (1961).
[8] P. Neri, G. Antoni, F. Benvenuti, F. Cocoda, and G. Gazze, *J. Med. Chem.*, *16*, 893 (1973).
[9] M. Tomida, T. Nakato, S. Matsunami, and T. Kakuchi, *Polymer*, *38*, 4733 (1997).
[10] T. Kakuchi, M. Shibata, S. Matsunami, T. Nakato, and M. Tomida, *J. Polym. Sci., Polym. Chem.*, *35*, 285 (1997).
[11] M. Tomida, M. Yabe, and Y. Arakawa, *Polymer*, *38*, 2791 (1997).
[12] Y. Abe, S. Matsumura, and J. Takahashi, *Oil Chemistry*, *35*, 167 (1986).
[13] H. Abe and K. Sakamoto, *Fragrance Journal*, *19*, (12), 47 (1996).