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ACID-CATALYZED COPOLYMERIZATION OF ASPARTIC ACID WITH ω-AMINO ACID.

— BIODEGRADABILITY AND Ca^{2+} CHELATING ABILITY OF POLY(ASPARTIC ACID -*CO*- ω-AMINO ACID) —

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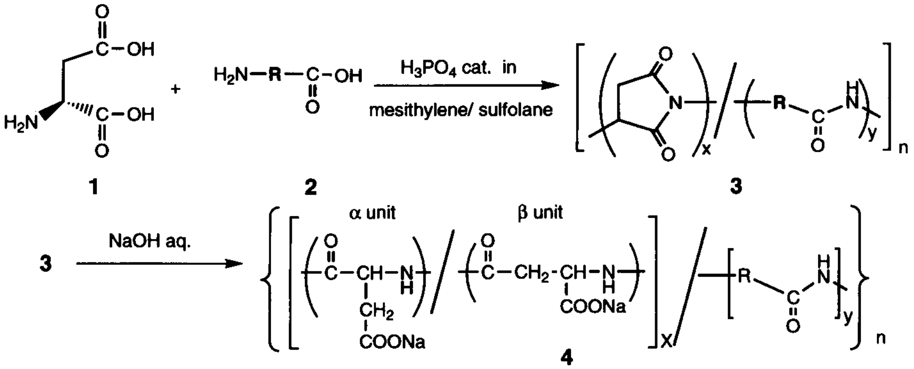
ABSTRACT

The polycondensation of L-aspartic acid (**1**) with various ω-amino acids (**2**) using phosphoric acid catalyst produced poly(succinimide-*co*-ω-amino acid)s (**3**), which was followed by alkali hydrolysis to poly(aspartic acid-*co*-ω-amino acid) (**4**). The Ca^{2+} chelating abilities of **4** depended on the content of comonomer unit in the copolymer and on the kind of amino acids. For the copolymer using 11-aminoundecanoic acid (**2d**) as a comonomer, the Ca^{2+} chelating ability was higher than that of poly(sodium acrylate). For poly(aspartic acid-*co*-6-aminocaproic acid) (**4c**), there was a tendency to increase according to the increase of 6-aminocaproic acid (**2c**) unit in the copolymer. The biodegradability of the copolymer in the case of **2c** as a comonomer, evaluated by TOC measurement, was 63 %, which was the highest degradability among the copolymers having different methylen length. The biodegradability of **4c** decreased with increasing the **2c** unit in **4c**.

INTRODUCTION

Polymers having carboxylic acid groups, such as poly(acrylic acid) and poly(methacrylic acid), are widely used as detergent builders, dispersants, and scale inhibitors but poorly decompose in nature [1]. On the other hand, poly-α-amino acid and its copolymer, which are very important for the modeling of proteins, are biodegradable and used as medical, cosmetic, fabric, and metal-absorbent materials [2-4]. In particular, poly-glutamic acid and poly-aspartic acid have been studied in terms of their synthesis and characterization [5, 6], e.g., poly-aspartic acid produced by the hydrolysis of poly(succinimide) has already been reported as a detergent builder [7-9].

Recently, we reported that high molecular weight poly(succinimide) was prepared by the polycondensation of L-aspartic acid (**1**) using catalytic amount of phosphoric acid and then poly(succinimide) was easily hydrolyzed to poly-aspartic acid with a high biodegradability [10]. However, the Ca^{2+} chelating ability, which is one important property for a detergent builder, was insufficient to use poly-aspartic acid in place of poly(acrylic acid) and poly(acrylic acid-*co*-maleic acid). In addition, we reported the synthesis of poly(succinimide-*co*-6-amino acid) with various comonomeric ratios and its hydrolyzed copolymer [11]. Thus it is interesting to elucidate the chelating ability of such copolymeric aspartic acid along with its biodegradable property.



R= $(CH_2)_m$: m=2 : 2a, 3a, 4a; m=3 : 2b, 3b, 4b; m= 5 : 2c, 3c, 4c;
m=10 : 2d, 3d, 4d; m=11 : 2e, 3e, 4e;

SCHEME 1

In this study, we report the synthesis of poly(aspartic acid-*co*-ω-amino acid) (**4**) by the polycondensation of **1** with various ω-amino acids (**2**) using an acid catalyst, followed by alkali hydrolysis, as shown in SCHEME 1. In addition, the effect of the methylene number in **2** unit on the Ca^{2+} chelating ability and biodegradability of **4** are investigated.

EXPERIMENTAL

Materials and Measurements

L-Aspartic acid (**1**) was obtained from the Mitsubishi Chemical Corporation (Japan), and 3-aminopropionic acid (**2a**), 4-aminobutylic acid (**2b**), 6-aminocaproic acid (**2c**), 11-aminoundecanoic acid (**2d**) and 12-aminolauric acid (**2e**) were purchased from Tokyo Chemical Industry Co., Ltd. ^1H NMR spectra were measured using a Bruker ASX300 NMR spectrometer. Molecular weight of copolymer was estimated using DMF solution containing 10 $mmol \cdot L^{-1}$ of LiBr as an eluent using a TOSOH HLC-8020 gel permeation chromatography (column: TSK-GEL (GMH_{HR}-M+G $2000H_{HR}$), detector: RI, standard: polystyrene).

Polycondensation of L-Aspartic Acid and ω-Amino Acids

A typical procedure for the polycondensation of **1** with **2c** is as follows: **1** (2.5 g, 19 mmol), **2c** (1.1 g, 8.1 mmol), and 85 %-*o*-phosphoric acid (0.25 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 h. Mesitylene 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-insoluble polymer (2.3 g; yield, 84 %).

Alkali Hydrolysis of Poly(succinimide-*co*-ω-amino acid)

To a 0.1 N NaOH aqueous solution (10 mL) was added **3** (100 mg) with stirring in an ice-bath for 20 min, and then the polymer was dissolved in the solution. This polymer solution was dialyzed against water for 24 h and then lyophilized.

Ca^{2+} Chelating Ability of Copoly Aspartic Acid

The Ca^{2+} chelating ability of polymer was determined using a calcium ion electrode and an ion meter in accordance with the description in a previous paper [11]. 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 min and the calcium ions in the solution were determined using a calcium electrode (Orion Model 93-20) and an ion meter (Orion Model 720A).

Biodegradability test

Biodegradability test was carried out based on OECD301C method (modified MITI TEST). The total organic carbon (TOC) of poly aspartic acid derivatives using standard activated sludge obtained from Chemical Inspection & Testing Institute, Japan, were measured at 25 ± 1 °C for 28 days, using the solution (300 mL), sludge concentration (30 mg/L) and polymer concentration (100 mg/L). Aniline was used as a standard.

RESULTS AND DISCUSSION

The copolycondensations of **1** and aliphatic ω-amino acids **2**s were carried out using *o*-phosphoric acid in mixed solvent of mesitylene/sulfolane (7/3 weight ratio) in molar feed of **1** / **2** (7 / 3) under reflux for 4.5 h. Poly(succinimide-*co*-ω-amino acid) (**3**) obtained were soluble in DMSO and DMF. Table 1 lists the results of the polycondensations of **1** with **2**. Polymer yields of **3** decreased with increasing the methylene number of comonomers. The compositions of succinimide units in all copolymers, which were determined from the ^{1}H-NMR spectra, tended to be higher than those of aspartic acid in the monomer feed. This means that the reactivity of **1** is higher than those of **2** during the acid-catalyzed polycondensation. For the weight-average molecular weight (M_w), which were determined by GPC in DMF using a polystyrene calibration curve, no homopolymer originated from **1** or **2** was produced because all GPC charts showed unimodal peaks. The highest

TABLE 1. Synthesis of Poly(succinimide-*co*-ω-amino acid) (**3**) and Poly(aspartic acid -*co*- ω-amino acid) (**4**) by Polycondensation of Aspartic acid (**1**) with ω-Amino acid (**2**).[a]

comonomer	3			4	
2	Yield (%)	succinimide unit in **3** (mol-%)[b]	$M_w(M_w/M_n)$[c]	water solubility[d]	α / β[b]
2a	94	70	6230 (1.75)	○	---
2b	92	80	21300 (2.12)	○	22 / 78
2c	84	76	14000 (1.64)	○	22 / 78
2d	80	79	14400 (1.70)	Δ	22 / 78
2e	79	71	21200 (1.41)	Δ	20 / 80

[a] Catalyst, H_3PO_4; solvent, mesithylene / sulpholane; temp., 180°C; time, 4.5 h; feed, (**1** / **2**)=(7 / 3).

[b] Determined by ^{1}H-NMR spectra.

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

[d] ○ means completely soluble in water and Δ means partially soluble in water.

M_w, 21300, was obtained for **2b**.

The hydrolysis of **3** was carried out using a 0.1 N NaOH aqueous solution in order to obtain poly aspartic acid derivatives. Polymers **3a**, **3b**, and **3c** were easily hydrolyzed to produce water-soluble polymers, whereas slightly amounts of water-insoluble polymers were observed for **3d** and **3e**. This means that higher hydrophobicity of **3d** and **3e** due to the long methylene chains of **2d** and **2e** units caused the decrease in solubility in alkali aqueous solution.

FIG. 1 shows the ^{1}H-NMR spectra of the sodium salt of the alkali-hydrolyzed copolymers **4b**, **4c**, and **4d** in deuterium oxide at 80 °C, respectively. The signals at 4.5 and 4.7 ppm and at 2.5 - 2.8 ppm were assigned to the methine and methylene protons of the aspartic acid unit, respectively. The assignment of comonomer unit were represented in FIG. 1. We confirmed that the hydrolysis of **3** completely proceeded to yield poly(aspartic acid-*co*-ω-amino acid) (**4**), because the signals at 4.4 - 4.6 and 5.1 - 5.3 and at 2.4 - 3.0 and 3.1 - 3.2 ppm due to the methine and methylene protons of the succinimide unit, respectively [11], were disappeared. The two chemical shift values of the methine proton were caused by the different ring-opening manner, i.e., α- and β-openings (SCHEME 1). The ratio of α-and β-openings, which were determined by the area ratio of the ^{1}H-NMR spectrum, is listed in TABLE 1. For every **4**, the ratio was almost constant as ca. 1 / 4, which is indicating that the β-

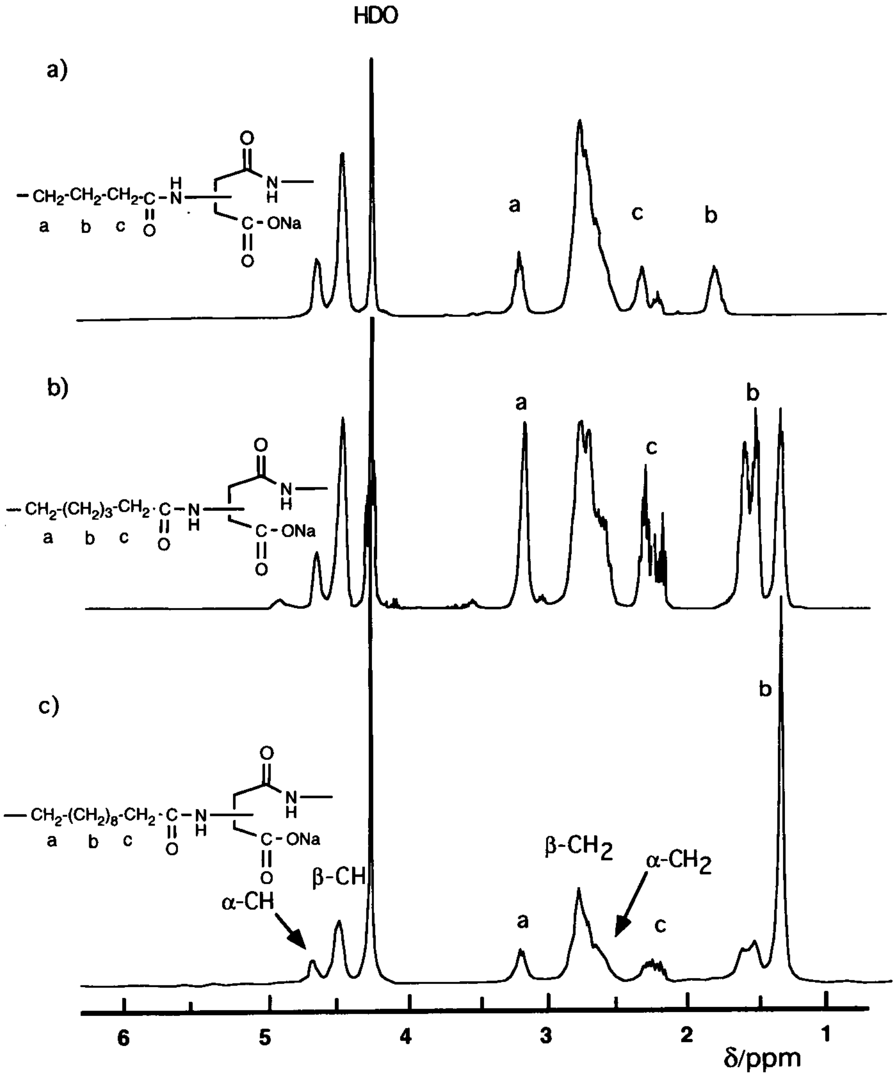


FIG. 1. ^{1}H-NMR (300 MHz, D_2O, 80 °C) spectra of poly(aspartic acid-*co*-ω-amino acid) **4b** (a), **4c** (b), and **4d** (c).

opening predominantly occurred rather than the α-opening. The ratio of α- and β-openings was also similar to that of poly-aspartic acid. This means that the ring-openning manner of succinimide is not affected by the kind of comonomeric unit on alkali hydralysis.

In order to estimate the property of detergent builder, a Ca^{2+} chelating experiment was carried out at pH 10, which is the actual condition of washing. FIG. 2 shows the Ca^{2+} chelating ability of copolymers **4** having different methylene number units. The chelating ability tended to increase according to the increase in the methylene number of ω-amino acid. The highest ability was obtained for the copolymer using 11-aminoundecanoic acid, **4d**, which is higher than that of poly(sodium acrylate) (**SPA**) with a M_w of 14000.

The biodegradation of poly-aspartic acid derivatives **4** were carried out using activated sludge and the biodegradability was evaluated by the value of total organic carbon (TOC). FIG. 3 shows the TOC values for various aspartic acid derivatives **4**. The biodegradability varied by changing the comonomeric unit, and in the case of poly(aspartic acid-*co*-6-aminocaproic acid) (**4c**) the highest value was obtained as TOC = 63 %.

Furthermore, in order to study the effect of the molar ratio of aspartic acid and ω-amino acid units, **4c** with the different copolymer composition of aspartic acid and 6-aminocaproic acid units, which were derived from poly(succinimide-*co*-6-aminocaproic acid)s [10], were used

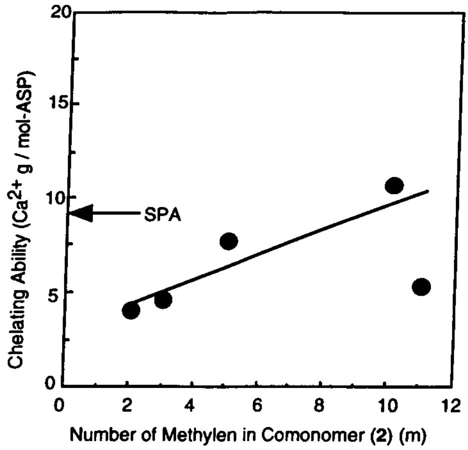


FIG. 2. Ca^{2+} chelating ability of Poly(aspartic acid-*co*-ω-amino acid) (**4**).

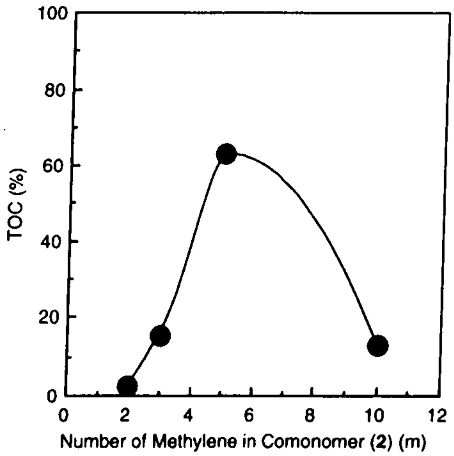


FIG. 3. Biodegradability of Poly(aspartic acid-*co* -ω-amino acid) (**4**).

TABLE 2. Biodegradability and Ca^{2+} Chelating ability of Poly(aspartic acid-*co*-6-aminocaproic acid) (**4c**)[a]

mol fraction of aspartic acid unit in polymer (%)	M_w	water solubility	Biodegradability [b] (TOC / %)	Ca^{2+} chelating ability [c] (Ca^{2+} g / mol-ASP)
100	64000	○	82.0	5.75
90	24000	○	78.2	6.25
76	14000	○	61.6	7.68
63	10000	○	27.7	9.70
35	4000	Δ	45.5	12.3
20	---	Δ	---	10.77

[a] Catalyst, H_3PO_4.; solvent, mesithylene / sulpholane; temp., 180°C; time, 4.5h. See reference[10].

[b] Using activated sludge; period, 28 days; temp., 25±1°C.

[c] *Using calcium-ion electrode*; PH,10; *temp.*, 30°C.

(TABLE 2). TOC decreased with decreasing the mol fraction of aspartic acid unit in **4c**. The association between a certain enzyme in activated sludge and a copolymer may be affected by the sequence and conformation of the copolymer, causing the difference in biodegradability. However this needs to further elucidate.

Although the copolymer with 20 mol-% of aspartic acid unit was not completely soluble in a NaOH solution, the Ca^{2+} chelating ability of **4c** increased with decreasing the aspartic acid unit in copolymer, as listed in TABLE 2. The chelating ability of copolymer with 35 mol-% of aspartic acid unit was almost twice as much as that of poly-aspartic acid. The chelating ability of **4c** with 63 mol-% of aspartic acid unit was similar to that of **SPA** with a M_w of 14000, and the ability of **4cs** with 35 mol-% or below of aspartic acid unit were higher than that of **SPA**. These results indicated that the chelating abilities depend on the kind of ω-amino acids and the content of aspartic acid unit in the copolymer. Therefore, the distance between two carboxylic acids in the polymer chain should act an important role on the Ca^{2+} chelating ability as well as biodegradability.

Conclusion

The polycondensation of aspartic acid with various ω-amino acids using *o*-phosphoric acid proceeded without gelation to form the organic solvent soluble copolymer, poly(succinimide-*co*-ω-amino acid). Poly(aspartic acid-*co*-ω-amino acid)s were obtained by the alkali hydrolysis of poly(succinimide-*co*-ω-amino acid). The Ca^{2+} chelating abilities increased with increasing hydrophobicity of poly(aspartic acid-*co*-ω-amino acid). The biodegradability of poly(aspartic acid-*co*-6-aminocaproic acid), which was highest among the copolymers with different ω-amino acid, increased with increasing the mol fraction of aspartic acid unit in the copolymer. These results suggested that the copolymer consisting of aspartic acid and ω-amino acid has the possibility to apply them as a biodegradable detergent builder.

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REFERENCES

[1] T.Hayashi, H. Nishimura, K. Sakano, and Y. Tani, *Biosci. Biotech Biochem.*, 58, (2), 444 (1994).

[2] T. Ouchi, M. Shiratani, M. Jinno, M. Hirao, and Y. Ohya, *Macromol.Chem.,Rapid Commun.*, 14, 825 (1993).

[3] T. Hayashi, and M. Iwatsuki, *Biopolymers*, 29, 549 (1990).

[4] G. L. Jain, and A.R. Ray, *Macromol. Chem.*, 182, 2557 (1981).

[5] P. Neri, G. Antoni, F. Benvenuti, F. Cocoda, and G. Gazze, *J. Med. Chem.*, 16, 893 (1973).

[6] S. K. Walk, G. Swift, Y. H. Paik, K. M. Yocom, R. L. Smith,. and E. S. Simon, *Macromolecules*, 27, 7613 (1994).

[7] K. C. Low, and L. P. Koskan, *Polym. Mater. Sci. Eng.*, 69, 253 (1993).

[8] K. C. Low, A. P. Wheeler, and L. P. Koskan, *Hydrophilic Polymers: Performance with Environmental Acceptance*; J. E. Glass, Eds.; *Am. Chem. Soc., Adv. Chem. Ser.*, 248, 99 (1996),

[9] M. Tomida, T. Nakato, M.Kuramochi, M.Shibata, S. Matsunami, and T. Kakuchi, *Polymer,* 37, 19 (1996).

[10] T. Kakuchi, M. Shibata, S. Matunami, T. Nakato, and M.Tomida, *J. Polymer. Sci.,* 35, 285 (1997).

[11] Abe, Y., Matsumura, S. and Takahashi, J., *Oil Chemistry*, **35**, (3), 167 (1986).