Relationships between Structure and Properties of Poly(aspartic acid)s

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Received November 5, 1997; Revised Manuscript Received February 2, 1998

ABSTRACT: Various types of poly(aspartic acid)s, which were poly(α-L-aspartic acid), poly(α-D-aspartic acid), poly(*β*-L-aspartic acid), and poly(α,*β*-D,L-aspartic acid)s, were prepared, and their biodegradabilities using the OECD 301C method and calcium ion chelating abilities were measured to clarify the relationship between the structure of poly(aspartic acid)s and these properties. Distinct tendencies were found both between the number of amide protons and biodegradability and between the ratio of the dicarboxylic acid end groups to the dicarboxylic acid end group plus succinimide end group and biodegradability. The chirality of the aspartic acid unit and the type of amide linkage in poly(aspartic acid) had no apparent effect on the biodegradability of poly(aspartic acid). The result of repetitive biodegradability analyses for poly-aspartic acid suggested the complete biodegradation is possible. Regarding the calcium ion chelating ability, only the type of amide linkage affected the calcium ion chelating ability. Poly(α-aspartic acid) showed a higher calcium ion chelating ability than poly(*β*-aspartic acid) and poly(α,*β*-aspartic acid).

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 hardly recovered or collected after use. Of concern is the diffusion and accumulation of such nonbiodegradable water-soluble polymers in the earth's environment after their 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, biodegradable substitutes for nonbiodegradable polymers having carboxylic acid groups are really desired in terms of the earth's environment.

Poly(amino acid) having free carboxylic-acid groups, such as poly(aspartic acid) and poly(glutamic acid), is one of the candidates for the biodegradable water-soluble polymer. Poly(glutamic acid) is synthesized by the polymerization of *N*-carboxyglutamic acid anhydride without using microorganisms, i.e., the NCA method.[4−6] However, the NCA method has a cost disadvantage and a production problem, because phosgene or diphosgene is needed for the synthesis of *N*-carboxyglutamic acid anhydride, resulting in a high cost. In addition, glutamic acid produces a cyclic unimer, pyroglutamic acid, by heating, though copolymers based on glutamic acid are obtained by thermal polycondensation.[7−11]

In terms of the chemical industry, biodegradable substitutes must be produced at low cost and on an industrial scale. Recently, the biotechnological production of L-aspartic acid with a low price and on an industrial scale has been realized.[12,13] Moreover, the thermal polycondensation of aspartic acid is known to easily produce poly(aspartic acid), so that it has a great potential to resolve environmental problems, in particular, in the field of biodegradable water-soluble polymer.[14]

There is a lot of research about the production of poly(aspartic acid),[15−24] but only a little research has investigated the relationship between the polymer structure and its biodegradability and chelating ability in order to use PASP.[25,26] Their relationships are the subject of this paper.[27]

The structural analysis of PASP has been mainly investigated using spectroscopic methods. Matsuyama et al. reported that the ratio of the α- and *β*-amide units in the PASP backbone was determined using ^{1}H NMR spectroscopy.[28] Pivacova et al. reported the ratio and distribution of the α- and *β*-linkages on the basis of ^{13}C NMR analysis.[29,30] We have already reported the detailed structure of thermally prepared polysuccinimide (PSI) prepared by a variety of methods.[31]

The relation between the PASP structure and its biodegradability had been previously investigated to a limited degree. For example, Alford et al. suggested that the *β*-amide linkage in thermally synthesized PASP caused a decrease in biodegradability.[32] The thermally synthesized PASP showed a lower biodegradability of about 70%, whereas the PASP prepared using *o*-phosphoric acid was completely biodegraded. There is no significant difference in the ratio of α- and *β*-amide bonds and the stereoregularity for both PASPs. However, the amide linkage of PSI due to a branched structure was not confirmed by the NMR measurement in Alford et al., and there was a difference in the amount of the amide protons between the PASPs synthesized with and without *o*-phosphoric acid. Therefore, the existence of the branched structure in PASP should cause a difference in their biodegradabilities.[33,34]

On the other hand, the relationship between the PASP structure and its chelating ability has not been as yet investigated. One of the biggest applications of poly(acrylic acid) is as a detergent builder. The key

S0024-9297(97)01629-X CCC: $15.00
Published on Web 03/12/1998

performance of a detergent builder is chelating ability, especially, calcium ion chelating ability. The research of the relationship between the PASP structure and its chelating ability is very important to substitute poly(acrylic acid) in the field of a detergent builder.

In this paper, various PASPs including several model polymers were prepared using various methods and evaluated regarding their biodegradabilities and calcium ion chelating abilities, including L- and D-aspartic acid and the oligomers of L-aspartic acid, and the relationship between their structure and properties is discussed more clearly.

Experimental Section

Materials and Measurements. L-Aspartic acid (ASP) was obtained from the Mitsubishi Chemical Corp. D-Aspartic acid, L-aspartic acid dimer, L-aspartic acid trimer, L-aspartic acid tetramer, L-aspartic acid pentamer, 85% *o*-phosphoric acid, mesitylene, sulfolane, *N*,*N*-dimethylformamide (DMF), sodium hydroxide, and deionized water were commercially obtained and used without further purification.

The molecular weight of PSI was estimated in DMF containing 20 mmol/L LiBr by gel permeation chromatography (GPC) (column, Plgel 5 mm MIXED-C × 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 31.2 g/L of $NaH_2PO_4 \cdot 2H_2O$ by liquid chromatography (LC) (column, Shim-pack ISC-07/S1504; detector, UV 210 nm). NMR spectra were obtained using a JEOL JNM-GSX400 spectrometer operating at 400 and 100 MHz for the ^1H and ^{13}C NMR measurements, respectively. Sample solutions were prepared by dissolving 100−200 mg of polymer in 0.6 mL of DMSO-d_6 in 5 mm NMR tubes. All spectra were recorded at 60 °C, and tetramethylsilane was used as the internal standard.

Preparation of Poly(aspartic acid). (a) Polycondensation of L-Aspartic Acid without Solvent and Acidic Catalyst. This procedure is basically described in the previous paper.[19,20]

A 200 mL four-necked round-bottom flask equipped with a thermometer, a cooler, a mechanical stirrer, and a N_2 inlet was charged with ASP (100 g, 0.752 mol). The mixture was heated at 260 °C for 6 h under a N_2 atmosphere. The conversion of ASP was 96 wt %. The product was washed several times with water (200 mL) until it was neutral. The residue was washed with methanol (200 mL) and dried at 85 °C under reduced pressure to yield PSI. The residual ASP was not detected in PSI.

The hydrolysis of PSI was carried out as follows: To a 100 mL beaker with a stirring bar were added PSI (3 g) and a solution of sodium hydroxide (1.4 g, equiv per succinimide residue), and deionized water (20 mL) under ice cooling. After the mixture was stirred for 1 h, the pH of the solution was adjusted by the addition of 35% aqueous HCl. The solution was poured into methanol (300 mL), and then the precipitate was filtered and dried at 40 °C under reduced pressure to yield PASP.

(b) Acid-Catalyzed Polycondensation of L-Aspartic Acid without Solvent. ASP (100 g, 0.752 mol) and 85% *o*-phosphoric acid (75 mmol) were mixed for 15 min at room temperature using a blender (Oster Osterizer). The mixture was transferred to a 200 mL four-necked round-bottom flask equipped with a thermometer, a cooler, a mechanical stirrer, and a N_2 inlet. After heating at 200 °C for 7 h, the conversion of ASP of the reaction product was 99 wt %; then the reaction product was washed several times with water (200 mL) until it was neutral. The residue was washed with methanol (200 mL) and dried at 85 °C under reduced pressure to yield PSI. The residual ASP was not detected in PSI. The hydrolysis of PSI leading to PASP was carried out using the same procedure as described in (a).

(c) Acid-Catalyzed Polycondensation of L-Aspartic Acid without Solvent Using a Twin-Screw Extruder. The general procedure is as follows: With a blender (Kawata Super Mixer), ASP (37.6 mol) and 85% *o*-phosphoric acid (3.76 mol) were mixed for 5 min at room temperature under a N_2 atmosphere. The twin screw extruder (KRC Kneader (Kurimoto Ltd.), diameter = 50 mm, L/D = 13.2) was used for the polycondensation mixture. The temperature of the barrel was 260 °C, the rotation speed of the screw was 30 rpm, and the amount of PASP production was 1 kg/h (the average retention time was 16 min). The conversion of ASP was 99 wt %. The product (100 g) was washed several times with water (200 mL) until it was neutral. The residue was washed with methanol (200 mL) and dried at 85 °C under reduced pressure to yield PSI. The residual ASP was not detected in PSI. PASP was obtained by the hydrolysis of PSI using the same procedure as described in (a).

(d) Acid-Catalyzed Polycondensation of L-Aspartic Acid without Solvent under Reduced Pressure. This procedure is basically described in the previous paper.[18]

A mixture of ASP (0.752 mol) and 85% *o*-phosphoric acid (0.451 mol) in a 2 L eggplant shaped flask was heated at 180 °C for 3.5 h under reduced pressure (about 5 mmHg) using a rotary evaporator. The reaction system changed from syrupy heterogeneous liquid to a glassy solid, as the reaction proceeded. After completion of the reaction, the pale brown glassy product was homogeneously dissolved at a bath temperature of 120 °C by adding DMF (400 mL). The solution was poured dropwise into water (1.5 L) to produce a resinous precipitate, and then this was ground with a mixer and filtered under a reduced pressure to produce a powder product. The conversion of ASP was 98 wt %. The product was washed with water until the pH of the rinse was neutral. This was dried at 85 °C under reduced pressure to obtain a cake of PSI. The residual ASP was not detected in PSI. The hydrolysis of PSI was carried out with the same procedure as described in (a).

(e) Acid-Catalyzed Polycondensation of L-Aspartic Acid with Solvent. 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 by using a Dean−Stark trap with a reflux condenser. After 4.5 h, the solvent was removed, the conversion of ASP of the precipitate was 98 wt %, and 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. The residual ASP was not detected in the residue. The hydrolysis of PSI was carried out with the same procedure as described in (a).

(f) Synthesis of Poly(α-L-aspartic acid) and Poly(α-D-aspartic acid) Using the NCA Method. Poly(*β*-benzyl L-aspartate) and poly(*β*-benzyl D-aspartate) were prepared according to a previous paper[35] and then hydrolyzed for conversion into poly(α-L-aspartic acid) and poly(α-D-aspartic acid), respectively, using a procedure for which the chirality and α-amide linkage did not change.[36]

(g) Synthesis of Poly(α,*β*-D,L-aspartic acid) Using the NCA Method. Poly(*β*-benzyl L-aspartate) and poly(*β*-benzyl D-aspartate) were prepared according to a previous paper[35] and then hydrolyzed for conversion into poly(α,*β*-D,L-aspartic acid).[36]

(h) Synthesis of Poly(*β*-L-aspartic acid) by Ring-Opening Polymerization. The preparation of poly(α-benzyl L-aspartate) and its hydrolysis leading to poly(*β*-L-aspartic acid) were carried out according to methods described in previous papers[36,37] by Dr. Kohei Sanui and Mr. Jyun Haruhara of Sophia University, Department of Chemistry, Tokyo, Japan.

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

Table 1. Effect of Reaction Conditions on Molecular Weight of Polysuccinimide

run	solvent	catalyst[a] (mol %)	reacn temp (°C)	reacn time	equipment[b]	M_w[c]
1			260	6.0 h	F	9 000
2		50	180	3.5 h	F	70 000
3	M/S[d]	10	reflux	4.5 h	F	64 300
4		10	200	7.0 h	F	14 000
5		10	180	7.0 h	F	12 000
6		3	200	7.0 h	F	9 900
7[e]		10	260	16 min	E	24 000
8[e]		10	260	16 min	E	23 000
9[e]		5	260	16 min	E	24 000

[a] The catalyst is 85% *o*-phosphoric acid. [b] F is flask and E is KRC kneader (twin-screw extruder). [c] M_w is the weight average molecular weight of PSI. [d] Mixed solvent of mesitylene and sulfolane (7/3). [e] The rotation speed of the screw is 30 rpm in runs 7 and 9 and 120 rpm in run 8.

OM3001 coulometer (Ohkura Electric Co., Ltd.) and a Total Carbon Analyzer TOC-5000A (Shimadzu Corp.), respectively. Removed TOC was calculated from the difference between the amount of the total organic carbon before and after the evaluation of biodegradability.

Repetitive Biodegradability of Poly(aspartic acid). The repetitive biodegradability of PASP was measured using a procedure similar to the OECD 301C method (Modified MITI Test) described above. For the first run, a sample was treated with the standard activated sludge at 25 ± 1 °C for 28 days and then 5 mL of the sample solution was collected. After the removed TOC was calculated from the difference between the amount of TOC before and after the evaluation of the biodegradability, the PASP sample was added to the solution to maintain a PASP concentration of 100 ppm. The amount of added PASP was calculated from the residual TOC result. The second run of the biodegradability was then evaluated in the same way, and the same procedure was repeated for a total of five times.

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.[38] 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 ion electrode (Orion Model 93-20) and an ion meter (Orion Model 720A).

Results and Discussion

Analysis of the Polysuccinimide Structure. Various PSIs were synthesized using thermal polycondensation with or without a solvent and with or without *o*-phosphoric acid under various conditions. Table 1 lists the effect of the reaction conditions on molecular weights of the obtained PSIs, and the results of the NMR analysis of the respective PSIs are summarized in Table 2 according to our previous paper.[29] The amounts of amide protons and each end group are represented as the numbers per 100 monomeric units in PSI. The regularity estimated from the ^{1}H NMR analysis was almost equal to that from the ^{13}C NMR analysis. The amounts of amide protons and each end group are different for the procedures with or without the catalyst and/or the solvent (runs 1−6) but are independent of the equipment used in runs 4−9. On the other hand, the molecular weight (M_w) varies on changing the reaction procedures, which depend on the efficiencies of the procedures. The proposed structure of the thermally prepared PSI is shown in Figure 1.

Table 2. Amount of Amide Proton and End Groups in Polysuccinimide

		end group				
		succinimide		maleimide		
run	amide proton ^{1}H[a]	^{1}H	^{13}C[b]	^{1}H	^{13}C	dicarboxylic acid ^{1}H
1	11.8	6.0	7.1	0.9	0.9	4.1
2	0.9	n.d.[c]	n.d.	0.1	n.d.	0.8
3	3.3	n.d.	1.2	0.3	0.8	2.9
4	4.7	1.9	2.5	2.1	2.1	2.9
5	8.5	0.8	0.8	2.2	1.7	5.3
6	11.0	3.9	3.6	2.0	1.8	11.0
7	3.9	1.3	1.4	1.4	1.5	4.2
8	6.5	1.0	0.9	1.9	1.7	5.8
9	12.5	1.3	1.2	1.8	1.7	7.5

[a] The amount of the end group is calculated by ^{1}H NMR analysis. [b] The amount of the end group is calculated by ^{13}C NMR analysis. [c] The peak is not detected. [d] The amount of each end group shows the amount of each end group per 100 monomer units in polysuccinimide.

Figure 2 shows the change in the number of amide protons and end groups according to the polycondensation time for run 4. The amino group was not detected and the succinimide and maleimide end groups increased after the first 30 min of the polycondensation, whereas the dicarboxylic acid end groups and the amide protons decreased according to the polycondensation time. Because the decrease in the number of amide protons means an increase in the ring formation, i.e., the succinimide unit, the polycondensation proceeds at a relatively slow rate and its completion requires about 3 h. This result agrees with that for the ASP consumption. The changes in the succinimide and maleimide end groups show that the chain scission of the polymer occurs after the first 30 min of the polycondensation.

Relation between Irregular Structure of Poly(aspartic acid) and Biodegradability. In the case of a water-soluble polymer such as PASP, the polymer structure should greatly affect the biodegradability. For PASP, the factors that are expected to affect the biodegradability are (1) irregular structures such as polymer branching and polymer end groups, (2) D- and L-structures and D- and L-sequence lengths, and (3) α- and β-amide linkages.

The primary structure of the polymer, especially the irregularities, which are expected to greatly effect the biodegradability, were investigated on the basis of the NMR analysis of PSI, as shown in Table 2. Because the irregular structures in PSI are preserved through the hydrolysis to PASP, the biodegradability of PASP should be discussed using the results of the analysis of the PSI structure. The maleimide end group should become a maleamic acid end group by the hydrolysis, though the carbon−carbon double bond still remains as an irregular structure in PASP. The succinimide end group similarly changes. Figure 3 shows the structural change of the end groups by hydrolysis. Table 3 lists the biodegradability estimated by the OECD 301C method (Modified MITI Test). After the polycondensation, the PSIs were washed with water to remove the residual ASP and the remaining catalyst. For all PASPs used, the D/L ratio was about 50/50 and the α/β-amide ratio was about 30/70, so that these structural effects are negligible. Both the BOD value and the removed-TOC value include the effect that PASP adsorbs onto the activated sludge, but the BOD values are lower than the removed-TOC values and the BOD values of the same sample are more scattered than the removed-TOC

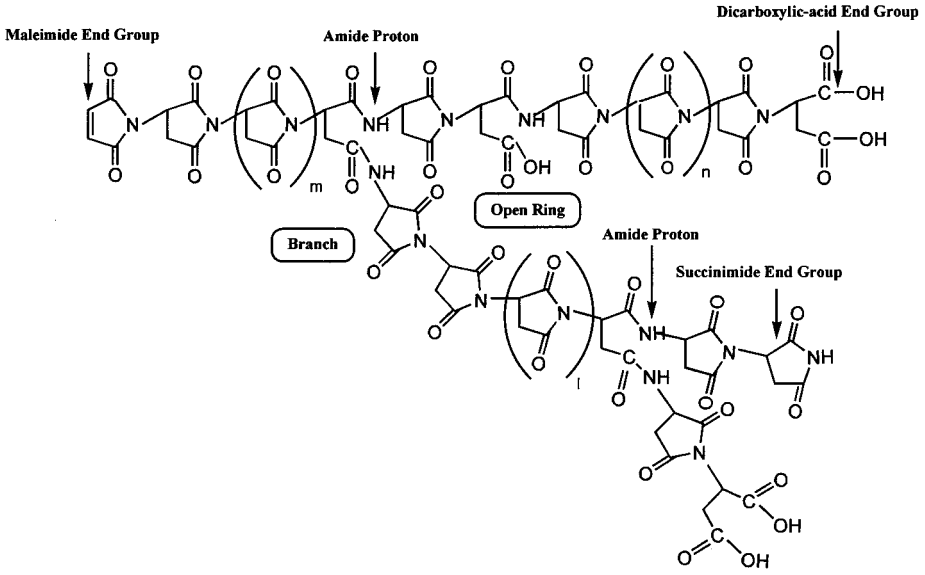

Figure 1. Structural image of polysuccinimide.

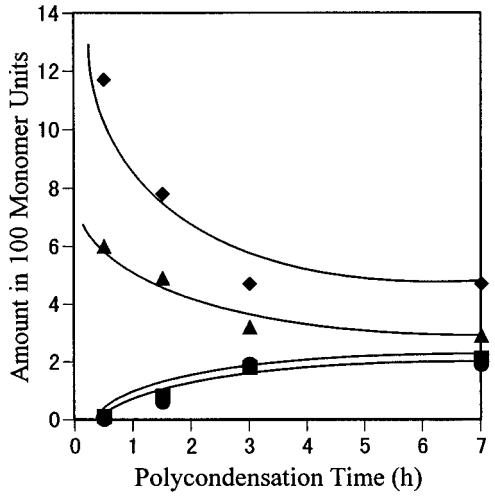

Figure 2. Changes of the amounts of the amide proton (◆) and the end groups, which are maleimide end group (■), succinimide end group (●) and dicarboxylic acid end group (▲), according to the polycondensation time.

values, which means that aspartic acids do not completely decompose to CO_2 and H_2O and must be incorporated into the biomass by microorganisms in the activated sludge during the measurement. This result indicates that the removed-TOC value should be used to evaluate the biodegradability of PASP, even if it includes the adsorption onto the activated sludge.

Figure 4 shows the relation between the amount of the amide protons and the biodegradability. Although the correlation is not strong, the amount of amide proton affects the biodegradability. The most important problem is to discuss the effect of the amide proton without being able to distinguish the branched structure and open-ring structure, because it is very difficult to determine the correct amount of the branched structure.

Figure 5 shows the relationship between the ratio of the end groups and the biodegradability. Because each polymer had a different molecular weight depending on each procedure, the numbers of the end groups in 100 monomer units are different. Therefore, the ratios of the end groups were used to discuss the effect of the end groups on the biodegradability. Natural poly(L-aspartic acid) should have amino and dicarboxylic acid

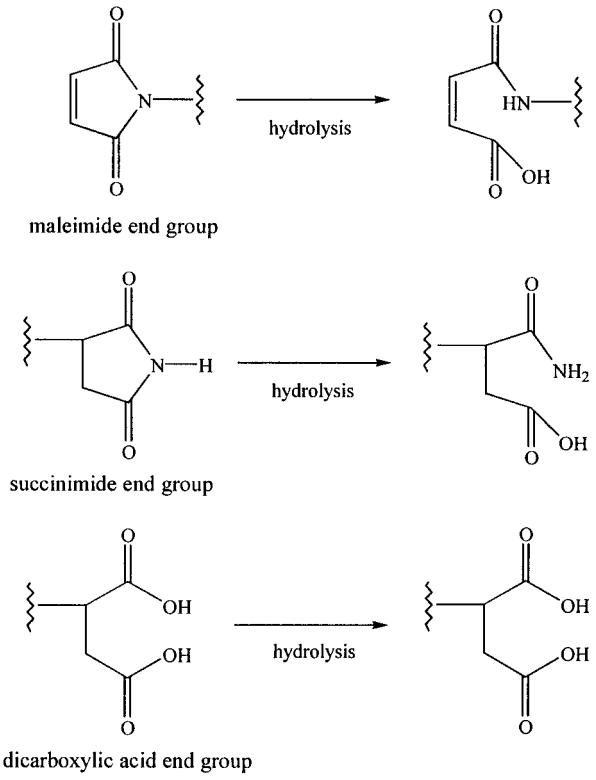

Figure 3. Structure changes of end groups by hydrolysis.

end groups. Because the amino end group was not detected in the synthetic PASP, the ratio of the dicarboxylic acid end group to the succinimide plus dicarboxylic acid end groups was used in Figure 5. This ratio focuses on the same side of the polymer (C-terminus), because the dicarboxylic acid end group changes to the succinimide end group during the polycondensation. These results indicate that the biodegradation mechanism of PASP proceeds through two types, i.e., the endo type as suggested from Figure 4 and the exo type from Figure 5. To maximize degradation, it is very important to control the amount and kind of the end groups and the amount of the amide linkage in PSI.

Relation between Chirality and Amide Linkage of Homopoly(aspartic acid) and Biodegradability.

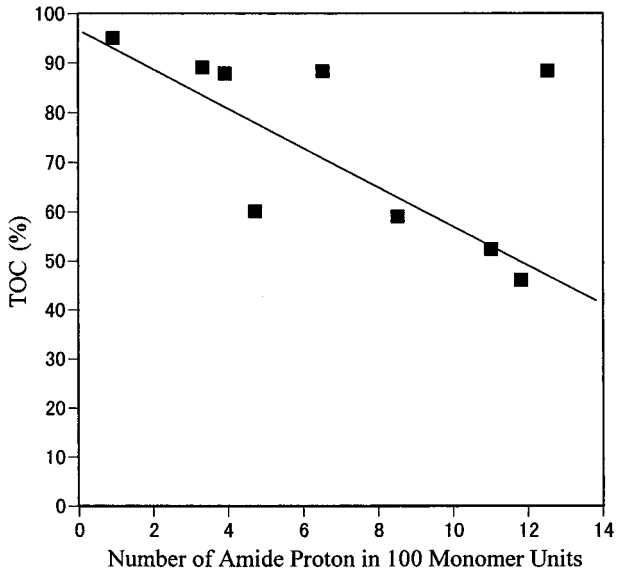

Figure 4. Relation between the number of amide protons and total organic carbon amount in various polysuccinimide procedures.

Table 3. Biodegradability of Poly(aspartic acid) by the Activated Sludge

run	removed TOC (%)	BOD (%)	run	removed TOC (%)	BOD (%)
1	46.0	26.0	6	52.3	50.1
2	95.0	81.0	7	88.3	80.3
3	89.0	82.0	8	87.8	81.1
4	60.0	58.2	9	88.3	83.2
5	59.0	51.7			

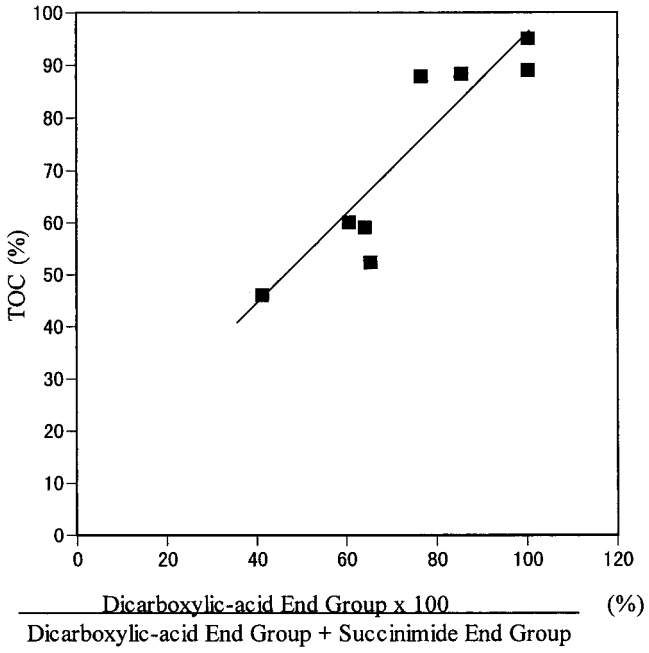

Figure 5. Relation between the ratio of the amount of dicarboxylic acid end groups to that of dicarboxylic acid end groups plus succinimide end groups, and total organic carbon amount in various polysuccimiinide procedures.

Two additional structural factors, the presence of (2) D- and L-structures and D- and L-sequence lengths and (3) α- and β-amide linkages, were investigated using D- and L-aspartic acids, the L-α-pentamer of L-aspartic acid, and several model polymers. The model polymers were produced by the *N*-carboxy amino acid anhydride (NCA) method and the ring-opening polymerization of the lactam. The results of the NMR and GPC analyses of the model polymers are summarized in Table 4. All model polymers had a linear structure by NMR analyses. Table 5 lists the biodegradabilities of the L- and D-aspartic acids, the 50/50 mixture of L- and D-aspartic acid, the α-pentamer of L-aspartic acid, and the model polymers shown in Table 4. Their biodegradabilities were uniformly very high, which suggests that the chirality of the monomeric units and the type of amide linkages in PASPs did not substantially affect the biodegradability of PASP.

Table 4. NMR and GPC Analyses of the Model Polymers

run	chirality	α	β	M_w (M_w/M_n)	synthesis
10	L	89.5	10.5	31 000 (1.5)	NCA[a]
11	D	89.5	10.5	31 000 (1.5)	NCA[a]
12	L/D = 50/50	27.3	72.7	34 000 (1.4)	NCA[b]
13	L	1.6	98.4	80 000 (2.2)	ring opening
3	L/D = 52/48	26.0	74.0	64 300 (1.9)	mixed solvent[c]

[a] HBr/acetic acid in trifluoroacetic acid was used to hydrolyze for removing benzyl group. [b] $NaOH/H_2O$ was used to hydrolyze for removing benzyl group. [c] Run 3 of Table 1.

Table 5. Effect of the Polymer Structure on the Biodegradability of Poly(aspartic acids)

	removed TOC (%)				
			polymer		
	monomer	pentamer	α	β	α/β = 30/70
L	93	97	91[a]	90[c]	
D	99		93[b]		
L/D = 50/50	97				89[d]
L/D = 50/50 (NCA)					87[e]

[a] Run 10 of Table 4. [b] Run 11 of Table 4. [c] Run 13 of Table 4. [d] Run 3 of Table 4. [e] Run 12 of Table 4.

The aspartic acid residue in the peptide is known to isomerize from the D- to the L-form, from the L- to the D-form, from the α- to the β-linkage, and from the β- to the α-linkage in various organisms.[39−42] It is also reported that D-aspartic acid is one of the major D-amino acids that exists in organisms. D-Aspartate oxidase (EC 1.4.3.1), aspartate racemase (EC 5.1.1.13), and D-aspartate aminotransferase were found in many microorganisms and animals, and many microorganisms capable of utilizing D-aspartic acid were also found.[43−51] It has been shown that D-aspartic acid is metabolized as well as L-aspartic acid by activated sludge.[34] The results in Table 5 indicate that microorganisms capable of decomposing D-aspartic acid exist in the activated sludge used.

For PASP, it is not clear that an enzyme directly decomposes the β-amide linkage. However, various enzymes, which can cleave the β-, γ-, and ω-amide linkages of the peptides containing β-alanine, glutamic acid, and lysine, are already known.[52−58] In addition, there is no substantial difference between poly(β-L-aspartic acid) and poly(α-L-aspartic acid). These facts suggest that enzymes capable of cleaving the β-amide linkage in PASP likely exist in nature.

Repetitive Biodegradation of Poly(aspartic acid). The biodegradability of PASP is high, but not 100%, even in the cases where the irregular structures in PASP are very low. This may be explained in two ways: First, an undetected structure in PASP, which cannot be decomposed by the activated sludge, may exist. Second, PASP may adsorb onto the activated sludge without degradation. If PASP has a structure that cannot be degraded by the activated sludge, the

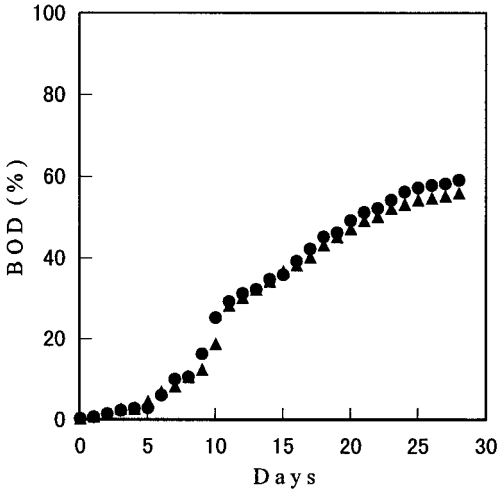

Figure 6. Degradation curves of BOD of first time (●) and fifth time (▲) in repetitive biodegradability.

Table 6. Result of Repetitive Biodegradability of Poly(aspartic acid) Produced with *o*-Phosphoric Acid Using Bulk Polycondensation

repetition	1	2	3	4	5
removed TOC (%)	60.0	66.5	65.9	64.5	60.3

undecomposed residues of PASP will accumulate. This means that thermally synthesized PASP is not acceptable in the environment.

To evaluate the biodegradability of PASP toward the activated sludge, the accumulation of the PASP residues was confirmed by the experiment in which the biodegradation of PASP was carried out using the same activated sludge system five times for five months. The same activated sludge was used, so PASP adsorption onto the activated sludge is postulated by the equilibrium. Therefore, this measurement should show the actual biodegradability of PASP. Table 6 and Figure 6 show the results for the polymer (run 4, Table 1). If PASP contains a nonbiodegradable portion, it will accumulate. If the accumulation occurs, the biodegradability should gradually decrease in proportion to the number of repeats. The removed-TOC values were almost equal for each of the five times. The degradation curves of the first time and the fifth time are very similar, and the degradation curves do not show any plateau; so there is no accumulation of PASP or its residues. Therefore, it appears that PASP is essentially 100% biodegradable.

Relation between the Structure of Poly(aspartic acid) and Calcium Ion Chelating Ability. The calcium ion chelating abilities of L-aspartic acid, the oligomers of L-aspartic acid, and PASPs were measured. The results are summarized in Table 7. On the basis of these results, the chirality of the aspartic acid unit does not affect the calcium ion chelating ability, but the type of amide linkage does affect it. The PASP with α-amide linkage shows the highest calcium ion chelating ability. The reason is postulated that the distance between the polymer backbone and the carboxylic acid affects the form of the complex between the calcium ion and carboxylic acid of PASP. The degree of polymerization also affects the calcium ion chelating ability. In the case of poly(acrylic acid), its calcium ion chelating ability increases according to the increase in the degree of polymerization up to about 100.[57] A similar tendency depending on the degree of polymerization was observed from the comparison of the calcium ion chelating abilities of L-aspartic acid pentamer and poly(α-L-aspartic acid).

Table 7. Effect of the Polymer Structure on the Calcium Ion Chelating Ability of Poly(aspartic acid) and Oligomers

	calcium ion chelating ability (g of Ca^{2+}/100 g of chelating agent)
L-aspartic acid	0.85
L-aspartic acid dimer	3.60
L-aspartic acid trimer	3.57
L-aspartic acid tetramer	5.05
L-aspartic acid pentamer	4.95
poly(α-L-aspartic acid)[a]	6.50
poly(α-D-aspartic acid)[b]	6.50
poly(α,β-D,L-aspartic acid)[c]	6.00
poly(α,β-D,L-aspartic acid)[d]	5.40
poly(β-L-aspartic acid)[e]	5.70

[a] Run 10 of Table 4. [b] Run 11 of Table 4. [c] Run 3 of Table 4. [d] Run 12 of Table 4. [e] Run 13 of Table 4.

Conclusions

Using structures determined by ^{1}H and ^{13}C NMR analyses of polysuccinimide produced by various methods, the relation between these structures and the biodegradability of poly(aspartic acid) produced by the hydrolysis of polysuccinimide was investigated. Distinct tendencies were found between both the number of amide protons and biodegradability and between the ratio of the dicarboxylic acid end groups to the dicarboxylic acid end group plus succinimide end group and the biodegradability. In contrast, the chirality of the aspartic-acid unit and the type of amide linkage in poly(aspartic acid) hardly affected the biodegradability of poly(aspartic acid). The results of experiments on the repetitive biodegradability of poly(aspartic acid) demonstrated no accumulation of the nondegradable residues. It was also found that only the type of amide linkage in poly(aspartic acid) affected the calcium ion chelating ability and poly(α-aspartic acid) showed the highest ability.

Acknowledgment. We gratefully acknowledge Dr. Kohei Sanui and Mr. Jyun Haruhara of Sophia University, Department of Chemistry, Tokyo, Japan, for supplying us with poly(α-benzyl L-aspartate) and poly(β-L-aspartic acid).

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MA971629Y