^{1}H and ^{13}C NMR Characterization of Poly(succinimide) Prepared by Thermal Polycondensation of L-Aspartic Acid[1]

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ABSTRACT: Poly(succinimide) was synthesized from L-aspartic acid at 260 °C for 6 h, and the microstructures of the polymer were analyzed in detail using ^{1}H and ^{13}C NMR spectroscopy. Several end groups, irregular structures, and byproducts were identified and quantified. Although C-termini such as dicarboxylic acid end groups and succinimide end groups occupy 10% of the monomer units in the polymer, an amino end group is not detected because it is probably reduced by the deamination which produces many irregular structures possessing a maleic or fumaric acid unit. The polymer chain is preferentially extended by the condensation of the amino group of a monomer and the dicarboxylic acid end group of the polymer. The presence of branched units and ring open amide units are shown by the results of DQF-COSY, a comparison of the amide proton signals, and the disparity between the sums of C- and N-termini. The stereoregularity of the investigated poly(succinimide) is low.

Introduction

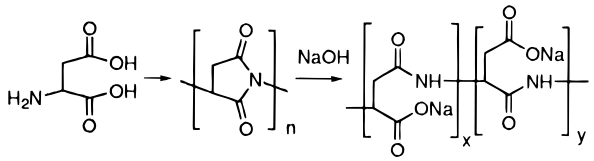
It is well-known that high molecular weight polymers with carboxylic acid side chains such as poly(acrylic acid) and acrylic acid/maleic acid copolymers show high performance as chelating agents and builders in synthetic detergents. Poly(acrylic acid) and acrylic acid copolymers do not decompose naturally. Accordingly, research on biodegradable polymers has become active recently.

Poly(amino acid)s with protein-like amide bonds are completely biodegradable. Sodium polyaspartate (SPA), a poly(amino acid) with carboxylic acid side chains, exhibits both biodegradability and functionality such as chelating ability and dispersibility. SPA offers the possibility to replace poly(acrylic acid) and related copolymers in many applications. SPA is commonly synthesized by the hydrolysis of poly(succinimide) (PSI) prepared by the thermal polycondensation of aspartic acid[2,3] as shown in Scheme 1.

The molecular structure of SPA has been mainly investigated using spectroscopic methods. Matsuyama et al.[4] determined the ratio of α- and β-amide units in the main chain by integrating the separated methine signals in the ^{1}H NMR spectrum recorded in the lower pD region. Pivcová et al.[5] evaluated the ratio of α- and β-amide units using the methylene signals in the ^{13}C NMR spectrum. They also analyzed the amide bond sequence using the amide carbonyl signals and concluded that the distribution of the α- and β-bonds was random.[6] Rao et al.[7] studied the tacticity effects for the amide carbonyl signals in the ^{13}C NMR spectrum. However, microstructures such as end groups and irregular structures have not been investigated in detail.

Wolk et al.[8] and Freeman et al.[9] investigated the structure−biodegradability relationship for SPA. The SPA synthesized with phosphoric acid degraded completely, while the one synthesized without phosphoric acid was about 70% degraded using activated sludge removability and CO_2 evolution tests. The two samples exhibited no significant difference in the ratio of α- and β-amide units and stereoregularity. They also analyzed PSIs, the intermediates to the SPAs, and concluded that the SPA synthesized without phosphoric acid has branching sites which would inhibit biodegradation. Although the mechanism of biodegradation for SPA has not been elucidated, if the exopeptidase, which hydrolyzes SPA from the chain end, play a significant part in degradation, the structures of the end groups and their concentration might also influence the biodegradability.

Scheme 1



In this paper, the microstructures of thermally prepared PSI are investigated in detail using ^{1}H and ^{13}C NMR spectroscopy. Several end groups, irregular structures, and byproducts are identified and quantified. The reaction mechanism of L-aspartic acid to form PSI and the irregular structures are discussed.

Experimental Section

Model Compounds. The following chemicals were used as model and authentic compounds for the comparison of NMR chemical shifts. *N*-cyclohexylmaleimide, succinimide, fumaric acid, maleic acid, and *N*-acetylalanylalanylalanine were commercially available and used without further purification. To prepare *N*-cyclohexylmaleamic acid, cyclohexylamine was added dropwise to the solution of maleic anhydride in toluene and the solution was heated at 60 °C for 2 h. The precipitated product was filtered out and dried in vacuum. Fumaramic acid was prepared from L-asparagine according to the method given in the literature.[10] The L,L-diketopiperazine of aspartic acid was synthesized in 70% yield by the dehydration of L-aspartyl-L-aspartic acid in DMSO-d_6 at room temperature for 30 days.

Polymers. L-Aspartic acid (Mitsubishi Chemical Corp., purity >99.9%) was used as a monomer. Succinic acid and 3,4-dimethylaniline (Wako Pure Chemical Industries) were used as comonomers.

The standard sample for structural analysis, PSI-T, was thermally polymerized without a phosphoric acid catalyst. A glass flask equipped with a condenser and a mechanical stirrer was charged with 270 g of L-aspartic acid and heated at 260 °C for 6 h under a nitrogen atmosphere. The obtained polymer (187 g, yield 95.2%) was not purified, and the weight-average molecular weight (M_w) was 9000.

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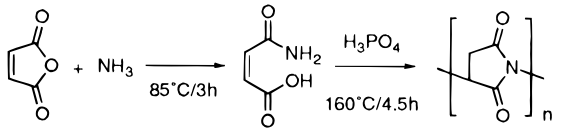
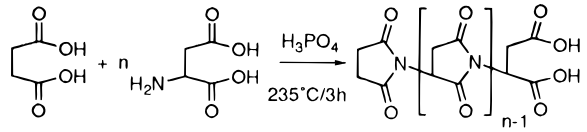
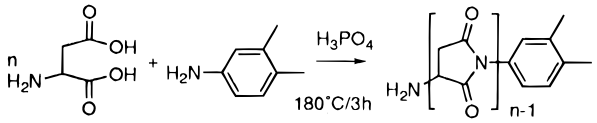
Scheme 2



Scheme 3



Scheme 4



A reference sample, PSI-P, was synthesized with a phosphoric acid catalyst.[11] L-Aspartic acid was mixed with 30 wt % phosphoric acid, and the mixture was heated at 180 °C for 3 h under reduced pressure. The reaction mixture was dissolved in *N*,*N*-dimethylformamide and the solution was poured into a large excess of water. The precipitate was filtered out and washed with water and then with methanol and finally dried in a vacuum. The M_w of the polymer was 1.0×10^5. A part of the PSI-P dissolved in *N*,*N*-dimethylformamide was added to a known amount of sodium hydroxide solution to hydrolyze the succinimide unit at a 10% level. The polymer was collected, washed with methanol, and dried at 40 °C in a vacuum.

Another sample, PSI-M, was synthesized from maleic anhydride and ammonia as shown in Scheme 2. After equimolar quantities of maleic anhydride and aqueous ammonia were heated at 85 °C for 3 h, the water was evaporated under reduced pressure. The resulting white substance was heated in mesitylene/sulfolane (7/3) with 0.1 equiv phosphoric acid at 160 °C for 4.5 h. The M_w of the polymer was 6000.

To prepare the PSI possessing many dicarboxylic acid end groups, L-aspartic acid and 10 wt % succinic acid was copolymerized at 235 °C for 3 h using 10 wt % phosphoric acid as shown in Scheme 3. The M_w of the polymer was 5700.

To prepare the PSI possessing many amino end groups, L-aspartic acid and 10 wt % 3,4-dimethylaniline was copolymerized at 180 °C for 3 h under reduced pressure using 30 wt % phosphoric acid as shown in Scheme 4. The M_w of the polymer was 5500.

Furthermore, three PSIs with polymerization times of 30, 90, and 180 min were prepared at 220 °C using 10 wt % phosphoric acid to follow the structural change with polycondensation time.

All reference PSIs were washed with water and then with methanol and were dried in a vacuum.

Gel Permeation Chromatography. The M_w was measured by gel permeation chromatography at 80 °C relative to polystyrene standards on two Plgel MIXED-C columns (Polymer Laboratories) connected in series. *N*,*N*-Dimethylformamide containing 0.02 M lithium bromide was used as the mobile phase with a flow rate of 1.0 mL/min, and a refractive index detector was used.

NMR Spectroscopy. All NMR spectra were obtained using a JEOL JNM-GSX400 spectrometer operating at 400 MHz for the ^{1}H nucleus and 100 MHz for the ^{13}C nucleus. Sample solutions for NMR were prepared by dissolving 100−200 mg of polymers in 0.6 mL of DMSO-d_6 in 5 mm NMR tubes. All spectra were recorded at 60 °C unless otherwise mentioned, and tetramethylsilane was used as the internal standard. The conditions for ^{1}H NMR were a 40° pulse angle, a 30 s delay between pulses, a 5.0 kHz spectral width, 32 K data points, and 32 scans. The conditions for ^{13}C NMR were a 60° pulse angle, a 15 s delay between pulses, a 22.0 kHz spectral width, 32 K data points, and 15 000 scans. A few drops of 4% sodium deuteroxide or 20% deuterium chloride were added to the DMSO-d_6 solution of PSI-M and then the ^{1}H and ^{13}C NMR spectra were obtained at 24 °C. The DEPT[12] spectra were collected with a 1.5 s recovery delay, a 22.0 kHz spectral width, 32 K data points, 24.0 μs 90° ^{1}H pulses, 9.2 μs 90° ^{13}C pulses, and 2000 scans.

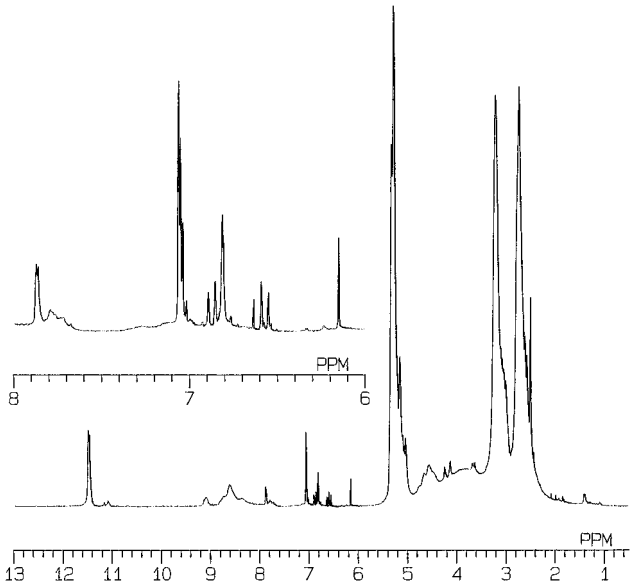


Figure 1. ^{1}H NMR spectrum of thermally prepared poly(succinimide) in DMSO-d_6 measured at 60 °C.

The DQF-COSY[13] data were acquired in the magnitude mode using 64 scans for each of the 256 t_1 increments, a 2.0 s recovery delay, spectral widths in f_1 and f_2 of 4.8 kHz, an acquisition time of 0.213 s, and 10.5 μs 90° ^{1}H pulses. Data were processed with shifted sine bell weightings and zero-filled to a 1K × 512 data matrix. The NOESY[14] data were acquired in the magnitude mode using the same parameters as in the DQF-COSY experiment except for 80 scans, a 1.8 s recovery delay, and a mixing time of 500 ms.

The ^{13}C-^{1}H heteronuclear correlation (HETCOR[15]) spectrum was acquired in the magnitude mode using 80 scans for each of the 256 t_1 increments, a 1.5 s recovery delay, spectral widths in f_1 and f_2 of 4.8 and 22.0 kHz, respectively, an evolution delay ($1/(2J)$) of 3.8 ms, an acquisition time of 0.093 s, 24.0 μs 90° ^{1}H pulses, and 9.2 μs 90° ^{13}C pulses. Data were processed with exponential weightings multiplied by a trapezoidal function and zero-filled to a 2K × 512 data matrix. The ^{13}C-^{1}H correlation via long range couplings (COLOC[16]) spectrum was acquired in the magnitude mode using 768 scans for each of the 128 t_1 increments, a 2.0 s recovery delay, spectral widths in f_1 and f_2 of 4.8 and 22.0 kHz, respectively, a long range evolution delay ($1/(2J)$) of 50 ms, an acquisition time of 0.093 s, 24.0 μs 90° ^{1}H pulses, and 9.2 μs 90° ^{13}C pulses. Data were processed with exponential weightings multiplied by a trapezoidal function and zero-filled to a 2K × 256 data matrix.

The 2D-INADEQUATE[17] spectrum was obtained using 500 mg of PSI-T dissolved in 3 mL DMSO-d_6 in a 10 mm NMR tube. The spectrum was acquired in the magnitude mode using 320 scans for each of the 128 t_1 increments, a 5.2 s recovery delay, spectral widths in f_1 and f_2 of 36 and 18 kHz, respectively, an evolution delay ($1/(4J)$) of 5.0 ms, an acquisition time of 0.057 s, and 19.8 μs 90° ^{13}C pulses. Data were processed with exponential weightings multiplied by a trapezoidal function and zero-filled to a 2K × 512 data matrix.

Results and Discussion

The ^{1}H and ^{13}C NMR spectra of PSI-T are shown in Figures 1 and 2, respectively, and the assignments of PSI and byproducts are summarized in Table 1 and Figure 3. The assignments of the signals are obtained for the most part by a combination of the following: (1) analysis of the proton−proton, proton−carbon, and carbon−carbon connectivity in the 2D NMR spectra; (2) distinction of the methylene, methine, and quaternary

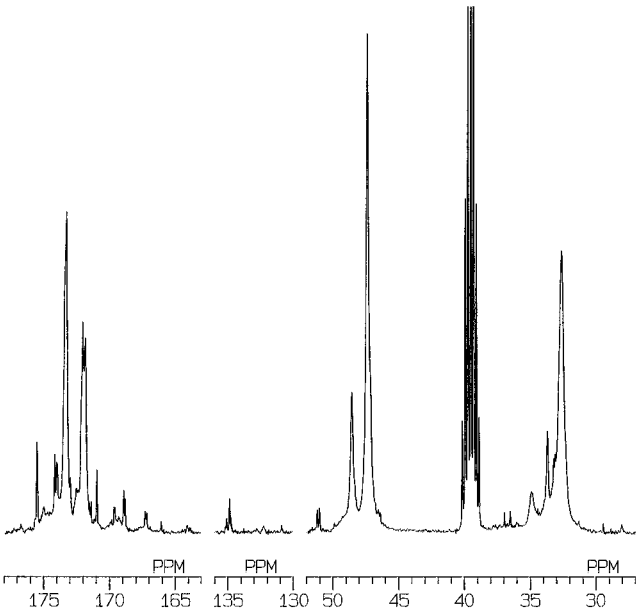


Figure 2. ^{13}C NMR spectrum of thermally prepared poly(succinimide) in DMSO-d_6 measured at 60 °C.

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts of Thermally Prepared Poly(succinimide) and Byproducts[a]

assign[b]	^{13}C chem shift (ppm)	^{1}H chem shift (ppm)	assign[b]	^{13}C chem shift (ppm)	^{1}H chem shift (ppm)
1	173.2		21	133.7	6.63
2	171.9		22		9.10
3	47.4	5.27	23	166.0	
4	32.6	2.72, 3.20	24	135.1	6.87
5	134.8	7.05	25	130.8	6.57
6	169.6		26		9.05
7	46.5	5.23	27	132.2	6.81
8		11.46	28	*c*	1.39
9	175.5		29	*c*	4.72
10	174.0		30[d]	167.3 (167.1)	
11	48.6	5.14	31[d]		7.87 (7.87)
12	33.7	2.60, 2.98	32[d]	51.0 (51.2)	4.23 (4.12)
13	170.9		33[d]	36.5 (36.9)	2.7 (2.7)
14	168.8		34[d]	171.5 (171.4)	
15	48.4	5.03	35	35	2.3−3.4
16	33.1	2.5, 3.07	36	49	4.3−4.8
17	*c*	4.14	37		7.6−9.3
18	*c*	2.62, 3.03			
19	129.8	6.15			
20	167.2				

[a] NMR spectra were obtained at 60 °C in DMSO-d_6 solution. [b] Numbering of each nucleus is shown in Figure 3. [c] Not assigned due to the low concentration or the overlapping of signals. [d] Chemical shifts of the L,L- and D,L- (in parentheses-) diketopiperazine of aspartic acid.

carbons using the DEPT spectrum; (3) internal consistency in the ^{1}H and ^{13}C NMR signal areas and quantitative consistency between the ^{1}H and ^{13}C NMR signal areas; (4) comparison of the chemical shifts of PSI with those of the model compounds; (5) changes in the chemical shifts with the gradual addition of deuterium chloride or sodium deuteroxide.

Structure of the Main Chain. The protons at 2.72 and 3.20 ppm are directly attached to the methylene carbon at 32.6 ppm and the proton at 5.27 ppm to the methine carbon at 47.4 ppm in the DEPT and HETCOR spectra shown in Figure 4. These three protons give cross-peaks to one another in the DQF-COSY spectrum shown in Figure 5. The fact that the relative amount of amide protons is small negates the possibility of a polyamide structure and confirms that the main chain of the polymer is composed of a five-membered imide ring. The two imide carbons adjacent to the methine and methylene carbons are assigned to the peaks at 171.9 and 173.2 ppm, respectively, using the 2D-INADEQUATE spectrum shown in Figure 6.

End Groups. The proton at 7.05 ppm is directly bonded to the carbon at 134.8 ppm in the HETCOR spectrum and long-range coupled to the carbon at 169.6 ppm in the COLOC spectrum shown in Figure 7. The proton is not coupled to any other protons in the DQF-COSY spectrum. This olefinic CH part is thought to be magnetically equivalent and is assigned to the maleimide end group because the corresponding chemical shifts of *N*-cyclohexylmaleimide are 6.93, 134.3, and 170.9 ppm. From the relative signal areas, the carbon at 46.5 ppm is assigned to the methine in the penultimate monomer unit.

The exchangeable proton at 11.46 ppm is assigned to the imide proton of the succinimide end group because the corresponding chemical shift of succinimide is 11.0 ppm. The comparison of peak areas and DQF-COSY spectrum show that the methine proton of the succinimide end group resonates at 5.14 ppm and the methylene protons resonate at 2.60 and 2.98 ppm. The methine and the methylene carbons are assigned to the peaks at 48.6 and 33.7 ppm, respectively, in the HETCOR spectrum. The imide carbon C-9 at 175.5 ppm and one of the methylene protons have a cross-peak in the COLOC spectrum. The areas of the two peaks at 175.5 and 174.0 ppm are equivalent in all samples. Figure 8 shows the ^{13}C NMR spectra of PSI-M recorded with the addition of a few drops of 20% deuterium chloride into the DMSO-d_6 solution. The low-frequency shifts of 0.1 ppm explained by the deuterium isotope effect[18] are observed in both peaks at 176.0 and 174.5 ppm (24 °C). Therefore, another imide carbon, C-10, is assigned to the peak at 174.0 ppm.

Figure 9 shows the ^{13}C NMR spectra of PSI-M obtained with the gradual addition of a few drops of 4% sodium deuteroxide solution. Two carbonyl peaks with equal areas at 169.2 and 171.3 ppm (24 °C) show high-frequency shifts which are explained by the change in the degree of acid dissociation.[19] In addition, both peaks are significantly enhanced in the ^{13}C spectrum of the copolymer of L-aspartic acid and 10% succinic acid (data not shown). Consequently, we assign these peaks to the dicarboxylic acid end group. The carbons C-13 at 170.9 ppm and C-14 at 168.8 ppm are assigned from the correlation to the methylene proton at 2.5 ppm and the methine at 5.03 ppm, respectively, in the COLOC spectrum of PSI-M (data not shown). The methine C-15 at 48.4 ppm and the methylene C-16 at 33.1 ppm are assigned from the high-frequency shifts with the sodium deuteroxide addition.

The two doublet protons at 6.57 and 6.87 ppm are coupled by 15.5 Hz, which indicates that the double bond has a trans configuration. The signal at 6.87 ppm is also correlated to the amide proton at 9.10 ppm in the NOESY spectrum shown in Figure 10. The corresponding chemical shifts of fumaramic acid are 6.51 and 6.87 ppm and the coupling constant is 15.6 Hz. We accordingly assign these peaks to the fumaramic acid end group. Although the double bond protons of *N*-cyclohexylmaleamic acid resonate at 6.23 and 6.40 ppm and the coupling constant of the olefinic protons is 12.8 Hz, no peaks are detected in the region of the spectrum for PSI-T. The maleamic acid end group with a cis configuration is not present.

The amino end group in PSI-T is not clearly identified probably due to its low concentration. To increase the

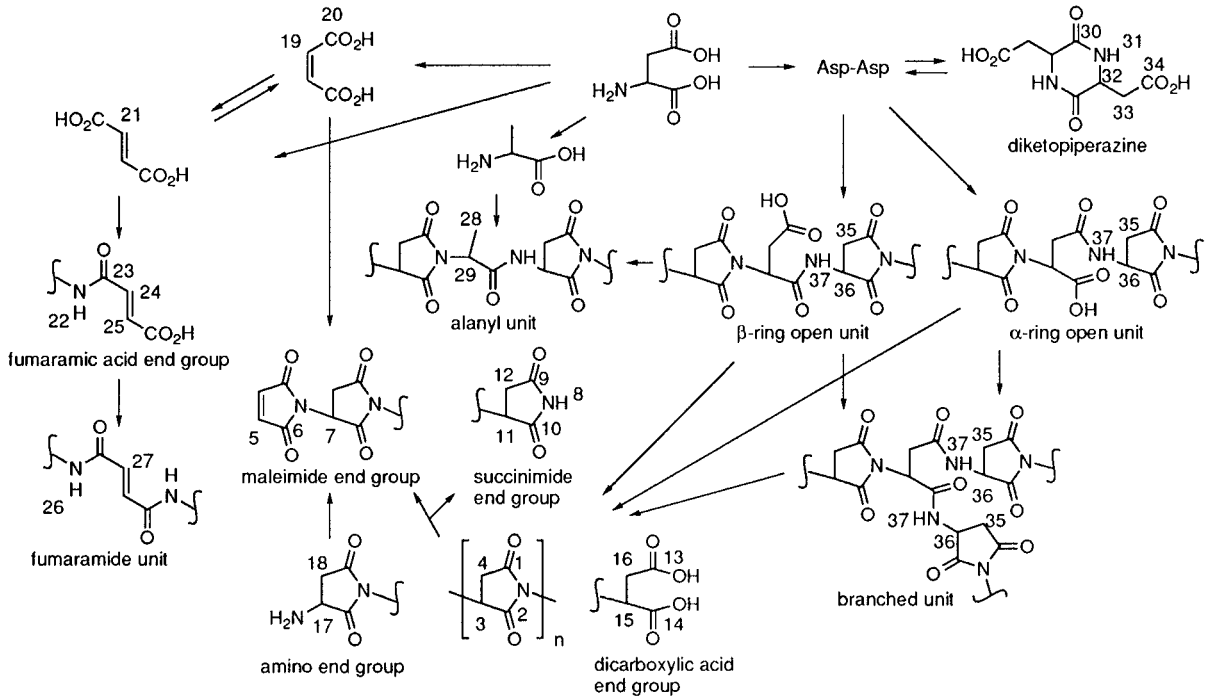


Figure 3. Numbering of each nucleus for NMR assignments and a proposed mechanism of L-aspartic acid to form poly(succinimide) and the irregular structures.

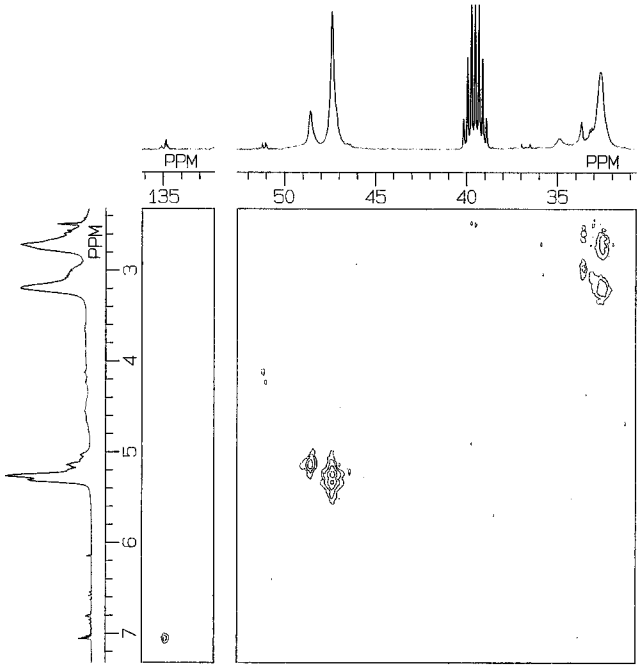


Figure 4. $^{13}C-^{1}H$ HETCOR spectrum of thermally prepared poly(succinimide) in DMSO-d_6 measured at 60 °C.

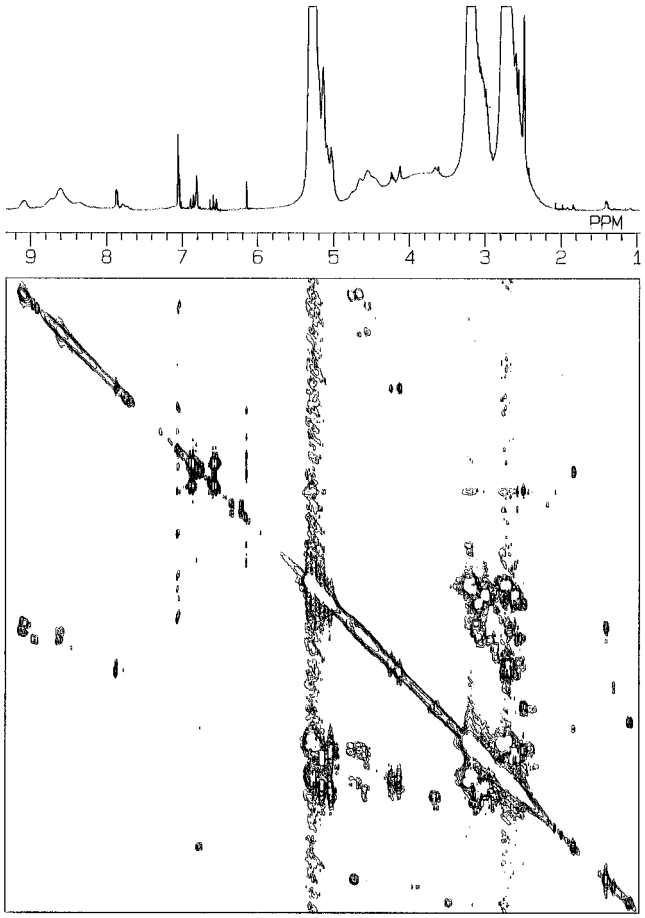


Figure 5. DQF-COSY spectrum of thermally prepared poly(succinimide) in DMSO-d_6 measured at 60 °C.

concentration of the amino end group, the copolymer of L-aspartic acid and 10 wt % 3,4-dimethylaniline was synthesized, and the DQF-COSY spectrum is shown in Figure 11. The methine proton at 4.14 ppm and two methylene protons at 3.03 and 2.62 ppm are ascribed to the amino end group. The amino proton is not observed probably due to rapid exchange with other protons.

Irregular Structures in the Main Chain. The proton at 6.81 ppm with no coupling partner in the DQF-COSY has a correlation to the amide proton at 9.05 ppm in the NOESY spectrum. The fumaramide unit and the maleamide unit with magnetically equivalent olefinic protons are considered as probable candidates. The chemical shift for the olefinic proton of the fumaramide unit is estimated from the chemical shift of fumaric acid (6.63 ppm) and those of the fumaramic acid end group (6.57 and 6.87 ppm) using the additivity rule for chemical shifts. The result is 6.81 ppm, and it is equal to the observed shift. Therefore, the signals are assigned to the fumaramide unit. The olefinic proton

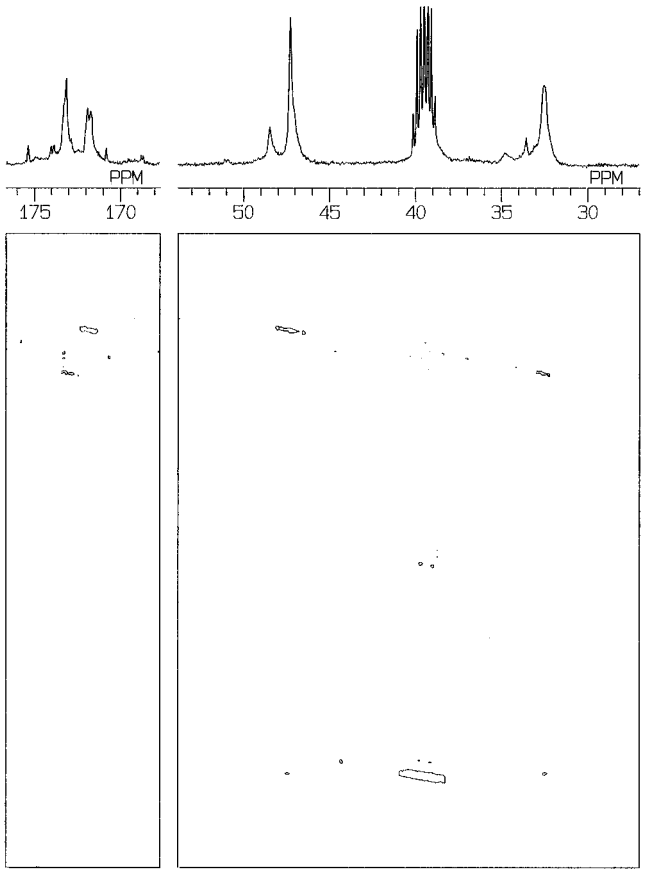


Figure 6. 2D-INADEQUATE spectrum of thermally prepared poly(succinimide) in DMSO-d_6 measured at 60 °C.

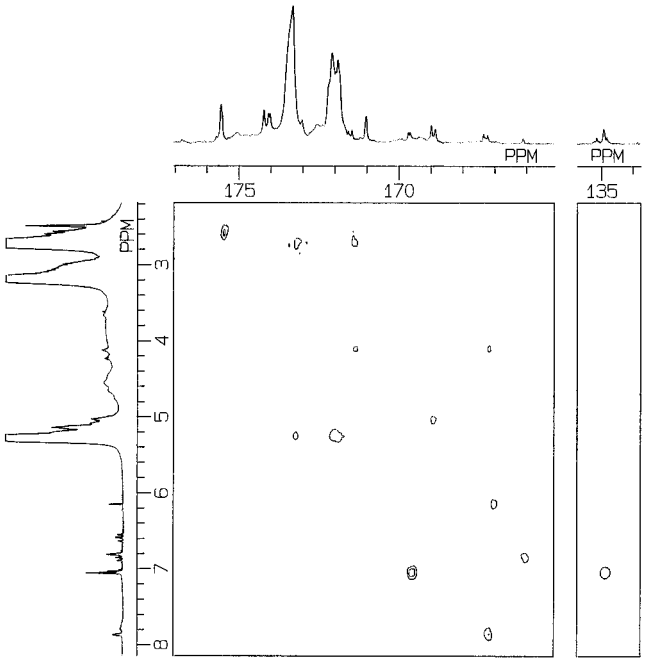


Figure 7. Expanded region of the $^{13}C-^{1}H$ COLOC spectrum of thermally prepared poly(succinimide).

of the maleamide unit is estimated to resonate at a lower frequency.

The doublet proton at 1.39 ppm is coupled to the quartet at 4.72 ppm by 7.3 Hz in the DQF-COSY spectrum. These peaks are assigned to the alanyl unit in the main chain. Although the observed methine shift (4.72 ppm) is at a higher frequency than that of the methine proton (4.29 ppm) of the central residue of *N*-acetylalanylalanylalanine, the difference between the

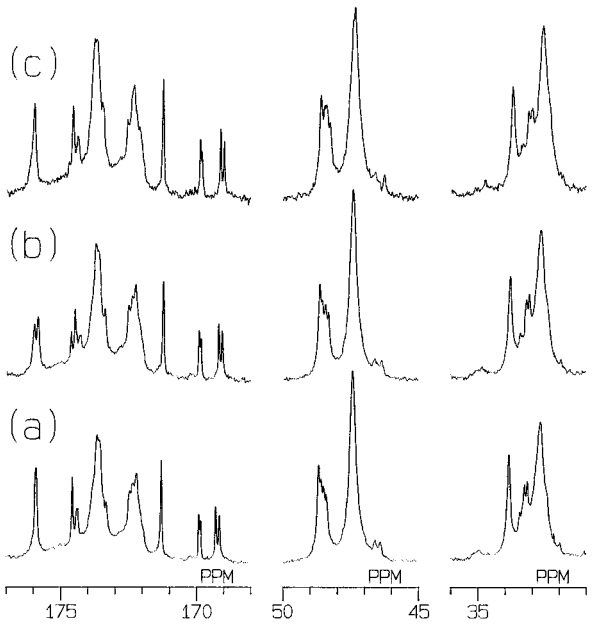


Figure 8. ^{13}C NMR spectra of PSI-M recorded at 24 °C with the gradual addition of (a) no drops, (b) 1 drop, and (c) 3 drops of 20% deuterium chloride into the DMSO-d_6 solution.

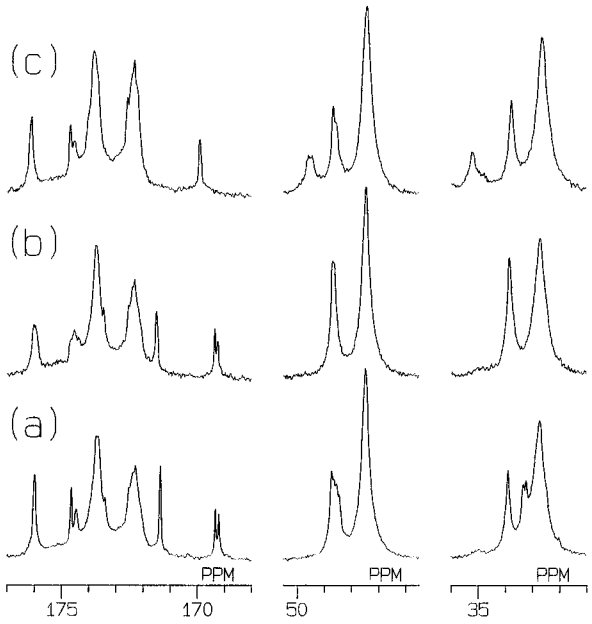


Figure 9. ^{13}C NMR spectra of PSI-M recorded at 24 °C with the gradual addition of (a) no drops, (b) 1 drop, and (c) 3 drops of 4% sodium deuteroxide solution.

observed shifts could be ascribed to the cyclic succinimide unit next to the alanyl residue.

The methine protons in the 4.3−4.8 ppm region are coupled to the amide protons at 7.6−9.3 ppm and to the methylene protons at 2.3−3.4 ppm in the DQF-COSY spectrum, which indicates the presence of −CONH−CH−CH_2− partial structures. As Wolk et al. postulated,[8] the branched and the ring open units shown in Figure 3 are the most probable structures. To distinguish the branched and the ring open units, the PSI possessing only the ring open units was synthesized by the partial hydrolysis of the imide ring of the PSI-P which has a high molecular weight and has few defect structures. The ^{1}H NMR spectrum of the 10% hydrolyzed PSI-P shows the signals of the ring open units in the regions 2.6−3.4, 4.3−4.8, and 8.2−9.8 ppm. Figure 12 shows the amide proton regions of PSI-T and 10% hydrolyzed PSI-P. The main signal at 8.6 ppm in PSI-T

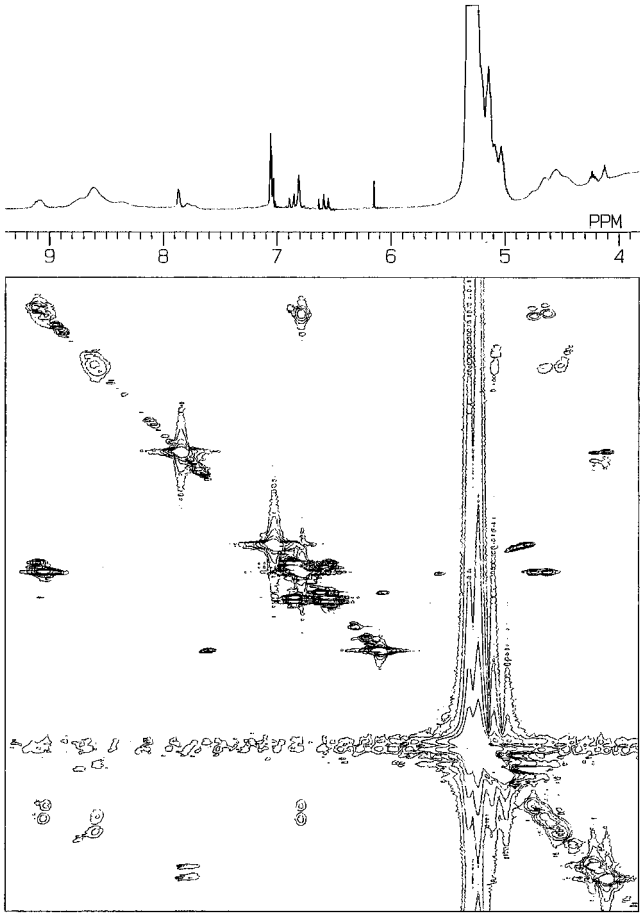


Figure 10. Expanded region of the NOESY spectrum of thermally prepared poly(succinimide).

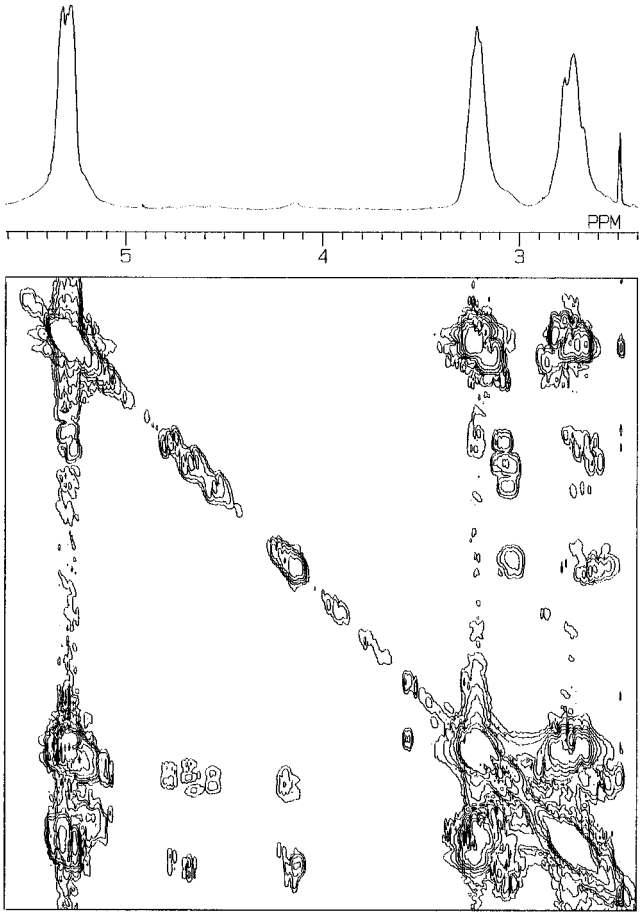


Figure 11. Expanded region of the DQF-COSY spectrum of the copolymer of L-aspartic acid and 10 wt % 3,4-dimethylaniline.

is slightly different from the one at 8.75 ppm of the ring open units in the 10% hydrolyzed PSI-P. If the 10% hydrolyzed PSI-P contains both the α- and β-ring open units, PSI-T includes both the branched unit and the ring open units. The ^{13}C peaks are not clearly assigned but the broad and overlapped peaks at 35, 48, and 168−176 ppm are considered as the corresponding carbons in these amide moieties.

Byproducts. The olefinic proton signals of maleic acid and fumaric acid are observed at 6.15 and 6.63 ppm, respectively. The proton at 4.23 ppm is coupled to the protons at 7.87 and 2.7 ppm in the DQF-COSY spectrum. The methine carbon at 51.0 ppm is bound to the proton at 4.23 ppm, and the methylene carbon at 36.5 ppm to the protons at 2.7 ppm in the HETCOR spectrum. These peaks are assigned to L,L(D,D)-diketopiperazine, a cyclic dimer of aspartic acid, because the chemical shifts coincide with those of the synthesized authentic sample. The amide carbonyl carbon at 167.3 ppm has correlations with the methine and amide protons, and the carboxylic acid carbon at 171.5 ppm has correlations with the methine and methylene protons in the COLOC spectrum. The protons at 2.7, 4.12, and 7.87 ppm and the carbons at 36.9, 51.2, 167.1, and 171.4 ppm have similar bond connectivity, and they are assigned to D,L-diketopiperazine of aspartic acid. A small amount of the diketopiperazine unit may be taken into the main chain of the polymer because broader signals are overlapped with those of the diketopiperazines.

Stereoregularity. The methine proton in the spectrum of PSI-P, which has a high molecular weight and has few defect structures, is composed of at least two main peaks split by 19.4 Hz. The splitting is not caused

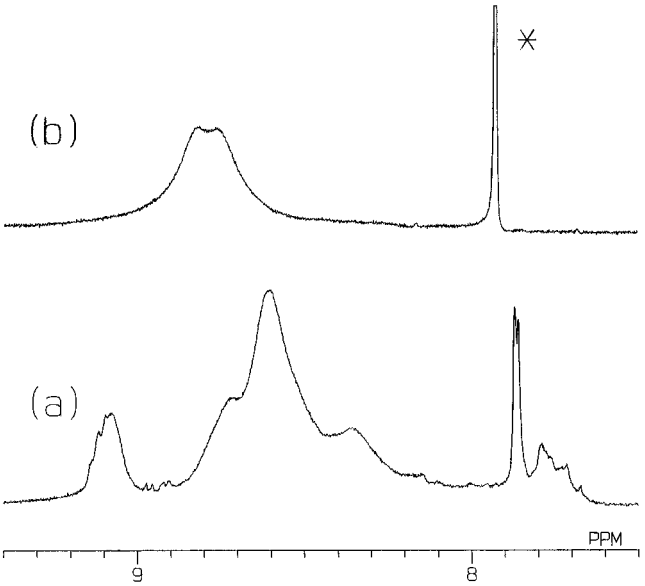


Figure 12. Amide proton regions of (a) thermally prepared poly(succinimide) and (b) 10% hydrolyzed PSI-P. The asterisk represents the amide proton of residual *N,N*-dimethylformamide used as the hydrolysis solvent.

by a spin coupling because the intensities of these peaks are not equal even though a weak coupling condition is fulfilled. The splitting can be ascribed to the difference in the stereoregularity caused by the partial racemization at the methine carbon. Although the peaks split by the methine-centered triad have not been completely assigned, the stereoregularity of the PSI is inferred to be low. This result is consistent with that reported by

Kokufuta et al.[20] They observed that the optical purity rapidly decreased with a rise in polycondensation temperature from 160 °C. PSI-T shows the same spectral feature. The fine splitting of C-1 and C-2 in the main chain and H-5, H-8, C-5, C-6, C-7, C-10, C-14, and C-16 in the end groups is thought to be caused in the same way.

Quantification for Minor Structures in PSI-T. The end groups, irregular structures, and byproducts of PSI per 100 monomer units are quantified on the basis of the above-described assignments. The methine proton signals are used to estimate the total monomer units in the main chain. The following proton signals are used to calculate the quantity of each minor structure: 7.6−9.3 ppm for the amide protons, H-5 for the maleimide end group, H-8 for the succinimide end group, H-25 for the fumaramic acid end group, H-27 for the fumaramide unit, H-28 for the alanyl unit, H-31 for diketopiperazines, H-19 for maleic acid, and H-21 for fumaric acid. The dicarboxylic acid end group is quantified using the C-14 signal and the total carbonyl carbon signals in the region of 163−178 ppm in the ^{13}C NMR spectrum. The spin-lattice relaxation times of the carbonyl carbons are 2.3 s for C-14 and 2.0 s for C-13 in the dicarboxylic acid end group, 2.2 s for C-10 and 1.7 s for C-9 in the succinimide end group, and 2.5 s for C-2 and 2.2 s for C-1 in the main chain. Therefore, the proton-decoupled ^{13}C NMR spectrum measured with a 60° pulse angle and a 15 s delay is used for quantification.

The calculated quantities of the end groups, irregular structures, and byproducts of PSI-T per 100 monomer units are as follows: the amide protons, 11.8; the maleimide end group, 1.0; the succinimide end group, 6.5; the fumaramic acid end group, 0.3; the fumaramide unit, 0.4; the alanyl unit, 0.2; diketopiperazines, 0.3; maleic acid, 0.08; fumaric acid, 0.02; the dicarboxylic acid end group, 4.1.

The amino, maleimide, and fumaramic acid end groups are N-termini in the polymer. The fumaramide unit is also considered as coupled two N-termini. On the other hand, the dicarboxylic acid end group and the succinimide end group are C-termini. The sum of the N-termini is 2.1 per 100 monomer units in PSI-T, and that of the C-termini is 10.6. If the major end groups and irregular structures are assumed to be assigned here, the great disparity between the sums of the C- and N-termini is ascribed to the significant amount of branched units, possibly including large ring structures. This result is consistent with that obtained from the comparison of the shapes in the amide proton signals previously mentioned.

To treat the amount of minor structures more quantitatively, if we assume that there is no ring open units left and all amide protons can be attributed to the branched unit, the quantitative relation of minor structures is expressed by the following equation:

$$N_{ae} + N_{ap}/2 + N_{me} + N_{fe} + 2N_{fu} = N_{de} + N_{se}$$

N_{ae} is the quantity of the amino end group per 100 monomer units; $N_{ap,}$ that of the amide protons; $N_{me,}$ that of the maleimide end group; N_{fe}, that of the fumaramic acid end group; N_{fu}, that of the fumaramide unit; N_{de}, that of the dicarboxylic acid end group; and N_{se}, that of the succinimide end group. The sum of the left-hand side of the equation is 8.0 and that of the right-hand side is 10.6 for PSI-T. The sum of the left-hand side is slightly less than that of the right-hand side even though the contribution of the ring open units to the amide protons is neglected. This discrepancy is probably caused in part by unidentified minor structures possessing units that invert the head-to-tail sequence of the monomer unit such as the diketopiperazine unit mentioned above and the iminodisuccinic acid unit.

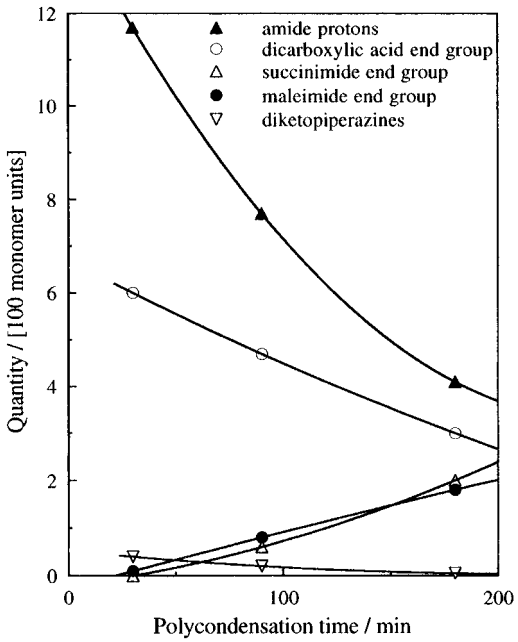


Figure 13. Change in the quantity of minor structures with polycondensation time. Poly(succinimide)s were prepared at 220 °C using 10 wt % phosphoric acid.

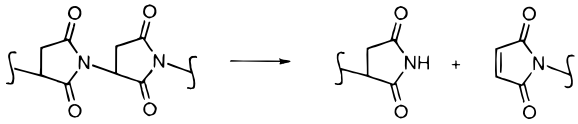
Reaction Mechanism. Figure 3 also shows a proposed mechanism of L-aspartic acid to form PSI and the irregular structures on the basis of the identified structures. The polymer extends its chain by intermolecular amide bond formation and cyclizes intramolecularly to form the succinimide ring with a loss of two water molecules per monomer unit. Figure 13 shows the change in the quantity of minor structures of PSI with polycondensation time; the PSIs were prepared at 220 °C using 10 wt % phosphoric acid. The amount of the amide protons and the dicarboxylic acid end group decreases as polycondensation progresses; this is consistent with the mechanism to form PSI.

Diketopiperazine is formed from a small part of L-aspartic acid during the early stage of polycondensation, 0.4 per 100 monomer units at 30 min in Figure 13, and then its concentration decreases while polycondensation proceeds. This result is consistent with that obtained by Harada.[21] He found that the reaction of the aspartic acid diketopiperazine diamide with malic acid at 180 °C for 2 h gave PSI, and assumed that diketopiperazine thermally formed as an intermediate was converted to the dipeptide again and the peptide reacted with another monomer or peptide to yield PSI.

The alanyl unit is assumed to be produced by the decarboxylation of β-carboxylic acid from the monomer or the ring open units. A β-alanyl unit produced by the decarboxylation of α-carboxylic acid is not identified in PSI-T.

The quantities of the maleimide end group and succinimide end group increase with polycondensation time in Figure 13. This variation suggests that the chain scission reaction which produces the same amount of maleimide and succinimide end groups simultaneously occurs during polycondensation as shown in Scheme 5. A similar scission reaction may happen to the branched unit and the ring open units. The quantity of the maleimide end group is less than that

Scheme 5



of the succinimide end group in PSI-T, which suggests that the maleimide end group further reacts at higher temperatures while the succinimide end group is stable or the succinimide end group is also produced through other routes.

The amino end group and the dicarboxylic acid end group are active growing points which form the amide linkage and lengthen the polymer chains. A relatively large amount of dicarboxylic acid end groups is present in PSI-T, 4.1 per 100 monomer units, while no amino end group is detected. It has not been detected even in the PSI polymerized under milder conditions, such as at 220 °C for 30 min with 10 wt % phosphoric acid. A small amount of the amino end group is detected only in the copolymer of L-aspartic acid and 10 wt % 3,4-dimethylaniline. We assume that the amount of amino end group is reduced by the deamination which produces maleic acid, fumaric acid, the fumaramic acid end group, and the fumaramide unit. Heating asparagine in phosphate buffer at 100 °C for 24 h produces fumaramic acid, while aspartic acid under the same conditions produces no fumaric acid.[10] The removal of the amino group of asparagine is due to the carboxamide function which has activated the adjacent methylene group. In the present study, it is likely that the aspartic acid peptide with a β-amide bond is more susceptible to the removal of the amino group than aspartic acid. Although the stability of the amino group of the aspartic acid peptide with an α-amide bond and the stability of the amino group connected to the succinimide ring are not clear, the amino end group of the homopolymers of aspartic acid is not detected under the present experimental conditions. Therefore, the condensation of the amino group of an aspartic acid monomer and the dicarboxylic acid end group of a polymer molecule is considered as a dominant step for the chain growth. Conversely, the reaction of the amino group of a polymer molecule with the dicarboxylic acid of an aspartic acid monomer is considered not to occur very frequently, and the possibility of bond formation between two polymer molecules also seems to be low.

The molecular weight of thermally polymerized PSI-T is less by 1 order of magnitude than that of PSI-P polymerized using phosphoric acid as the catalyst. The molecular weight of PSI-T is most likely lowered by the previously mentioned chain scission and deamination reactions.

Relation of PSI to SPA. Using the knowledge for the microstructures of PSI, we can predict the end groups and irregular structures of SPA, which is synthesized by the hydrolysis of PSI. The imide linkages are hydrolyzed to the α- and β-amide linkages under the usual hydrolysis conditions while the amide linkages are left intact. For example, the fumaramic acid end group and the fumaramide unit would remain unchanged, while the succinimide, maleimide, and amino end groups would become the corresponding end groups with acyclic amide linkages. Investigating these end groups and irregular structures of SPA and studying the structure−property relationships are future directions of our research.

Conclusions

The microstructures of thermally prepared PSI were analyzed in detail using ^{1}H and ^{13}C NMR spectroscopy. The maleimide, succinimide, dicarboxylic acid, and fumaramic acid end groups were identified. On the other hand, the amino end group and the maleamic acid end group were not detected. The branched unit, ring open amide unit, and alanyl unit were identified as irregular structures in the polymer. Maleic acid, fumaric acid, and diketopiperazines were formed as byproducts. The stereoregularity of the investigated PSI is inferred to be poor. The amino end group is probably reduced by the deamination which produces many irregular structures possessing a maleic or fumaric acid unit. The polymer chain is preferentially extended by the condensation of the amino group of a monomer and the dicarboxylic acid end group of the polymer.

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