End Group and Irregular Structure Analysis in Thermally Prepared Sodium Polyaspartate by ^{1}H and ^{13}C NMR Spectroscopy

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ABSTRACT: Sodium polyaspartate (SPA) was synthesized by the hydrolysis of poly(succinimide) (PSI) prepared by the thermal polycondensation of 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 and irregular structures which had most likely been produced by deamination and decarboxylation were identified and quantified. The presence of branched units was demonstrated by the detailed analysis of the methine proton region using the specially prepared model polymers. The number of the branched units was 3 per 100 monomer units, and the average number of monomer units between two adjacent branch points and between a branch point and an end group was estimated to be 13. The comparison of the quantities of the minor structures in SPA with those of the corresponding ones in PSI revealed that side reactions scarcely occur during the hydrolysis of PSI to SPA.

Introduction

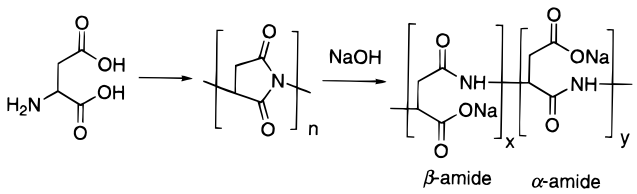
Poly(acrylic acid) and acrylic acid copolymers which are used as builders in detergents are not totally biodegradable, although poly(amino acid)s with protein-like amide bonds are completely biodegradable. Sodium polyaspartate (SPA), a poly(amino acid) with carboxylic acid side chains, exhibits both biodegradability and functionality as a builder. Therefore, SPA can be regarded to be more suitable than acrylic acid-based polymers as builders in the detergents, because the social concern for protecting the environment of rivers and lakes is gradually growing.

SPA is commonly synthesized by the hydrolysis of poly(succinimide) (PSI) prepared by the thermal polycondensation of aspartic acid[1,2] as shown in Scheme 1. The polymer chain of SPA is connected with α- and *β*-amide bonds. The ratio of the α- to *β*-amide units has been determined by integration of the separated methine signals in the ^{1}H NMR spectrum[3] and the methylene signals in the ^{13}C NMR spectrum.[4] Pivcová et al.[5] have also analyzed the amide bond sequence using the amide carbonyl signals and have concluded that the distribution of the α- and *β*-bonds is random. The optical purity of PSI decreases rapidly with a rise in the polycondensation temperature from 160 °C.[6] Rao et al.[7] have studied the tacticity effects on the amide carbonyl signals in the ^{13}C NMR spectrum of SPA. However, microstructures such as end groups and irregular structures have not been investigated in detail.

Wolk et al.[8] and Freeman et al.[9] have investigated the structure−biodegradability relationship for SPA synthesized with and without a phosphoric acid catalyst and have concluded that the SPA synthesized without phosphoric acid has branching sites which would inhibit biodegradation. The ^{1}H NMR signals of partial structures indicating the presence of a branching site or unclosed amide units in PSI have been observed; however, the signals of the branching site in SPA have not directly been observed.

To acquire some information on the polycondensation mechanism of aspartic acid, the end groups and irregular structures of thermally prepared PSI were closely investigated by NMR in our previous work.[10] The presence of the branched unit and unclosed amide unit is indicated but their quantity cannot separately be determined because the amide proton signals of the branched unit and unclosed amide unit as well as other ^{1}H and ^{13}C signals are severely overlapped.

Scheme 1



In this paper, the microstructures of thermally prepared SPA were investigated in detail using ^{1}H and ^{13}C NMR spectroscopy. Several model compounds were prepared for the identification of the end groups and irregular structures. The appropriate sample preparation and the choice of the temperature for the ^{1}H NMR measurement made it possible to remove the disturbance of the residual water signal and facilitate the interpretation of the spectrum. The structures of the minor components including the branching site were clearly determined. The relation of PSI to SPA concerning the structures of the minor components and their quantity was examined.

Experimental Section

Model Compounds. The following chemicals were used as model and authentic compounds for the comparison of NMR chemical shifts. Fumaric acid, maleic acid, L-aspartyl-L-aspartyl-L-aspartic acid (Asp-Asp-Asp), and DL-alanyl-L-asparagine 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 under vacuum. Fumaramic acid was prepared from L-asparagine according to the method given in the literature.[11]

Synthesis of PSIs. The preparation conditions and the weight-average molecular weight (M_w) of the samples are

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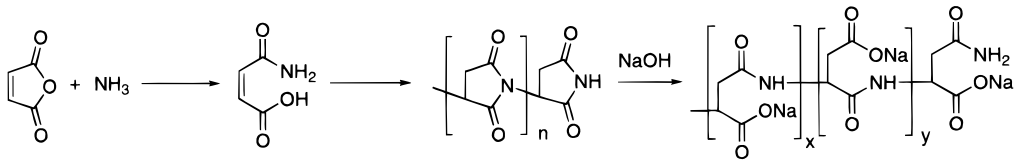
Table 1. Summary of Preparation Conditions and M_w of PSI and SPA[a]

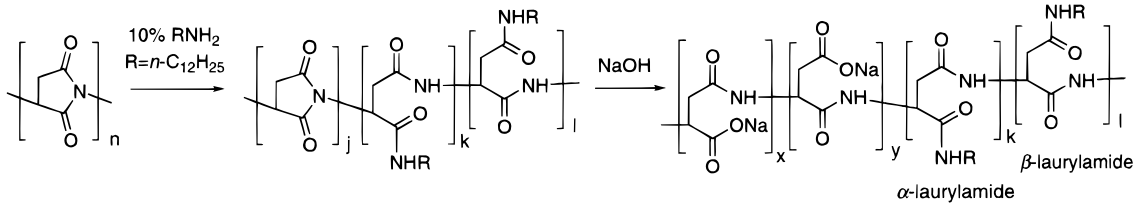
sample	reactants and their composition	catalyst	temp (°C)	time (h)	M_w of PSI	M_w of SPA
PSI, SPA−1	Asp	none	260	6	9000	11000
−2	Asp	H_3PO_4	180	3	100000	91000
−3	maleic anhydride/NH_3 = 1/1 (mol/mol)	H_3PO_4	160	4.5	6000	4300
−4	Asp/succinic acid = 9/1 (w/w)	H_3PO_4	235	3	5700	5700
−5	Asp/3,4-dimethylaniline = 9/1 (w/w)	H_3PO_4	180	3	5500	5500
−6	PSI−2/laurylamine = 9/1 (mol/mol)	none	24	6	100000	*b*

[a] The polydispersity of all samples is in the range 1.2−2.8. [b] Cannot be measured by the aqueous GPC column used.

Scheme 2



Scheme 3



summarized in Table 1. The standard sample for structural analysis, PSI-1, 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 (Mitsubishi Chemical Corp., purity >99.9%) and heated at 260 °C for 6 h under a nitrogen atmosphere. The amount of the obtained polymer was 187 g and the yield was 95%. The polydispersity (M_w/M_n) of the polymer was 1.6.

The synthesis of the following model PSIs and the confirmation of their structural features are described elsewhere.[10] High molecular weight PSI-2 was synthesized with a phosphoric acid catalyst.[12] PSI-3 with many succinimide end groups was synthesized from maleic anhydride and ammonia as shown in Scheme 2. PSI-4 possessing many dicarboxylic acid end groups was synthesized using 10 wt % succinic acid as an end-capping agent. PSI-5 possessing many amino end groups was synthesized using 10 wt % 3,4-dimethylaniline as an end-capping agent.

A model polymer possessing branched units, PSI-6, was prepared as shown in Scheme 3. A part of PSI-2 dissolved in *N*,*N*-dimethylformamide was partially aminolyzed with 0.1 equivalent of laurylamine at 24 °C for 6 h. The polymer was fractionated by precipitation with water, washed with methanol, and dried under vacuum. NMR analysis revealed that, relative to the aspartyl unit, 3 mol % of laurylamine was incorporated in the polymer. Cyclopentylamine was thought more appropriate for the preparation of the model polymer possessing the branched units, but it did not react with PSI under the same reaction conditions.

Hydrolysis of PSIs. A total of 2 g of finely ground PSI-1 powder was added by several steps to a 10 mL aqueous solution of 0.85 g (equivalent amount) of NaOH while the flask was cooled in an ice bath. After the last addition, the suspension was stirred for a further 1 h at room temperature until it became a clear solution. The solution was poured into a large excess of methanol, and the precipitated polymer was filtered out, washed with methanol, and finally dried at 45 °C for 12 h under vacuum. Other model polymers were hydrolyzed in a similar way.

A sodium aspartate oligomer connected with only a β-amide bond was prepared by the hydrolysis of SPA under rigorous conditions. The aqueous solution of a part of SPA-2 and an equivalent amount of NaOH were heated at 90 °C for 2 h. The solution was poured into a large amount of methanol, and the precipitate was filtered out, washed with methanol, and dried under vacuum.

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

The M_w of SPA was measured by aqueous GPC at 40 °C relative to poly(ethylene oxide) standards on TSKgel G6000PWxl and TSKgel G3000PWxl columns (Tosoh Corp.) connected in series using a 0.4 M sodium nitrate mobile phase and a flow rate of 1.0 mL/min, and a refractive index detector was used.

NMR Spectroscopy. Sample solutions for NMR measurement were prepared by dissolving 100 mg of polymers in 0.6 mL of D_2O in 5 mm NMR tubes. The polymers were once lyophilized from 1 mL of D_2O to reduce the residual water signal. The pD of the solution was adjusted to 8, if necessary, by sodium deuterioxide or deuterium chloride. All NMR spectra were obtained using a JEOL JNM-GSX400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C. All spectra were recorded at 24 and 60 °C unless otherwise mentioned, and sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 was used as an internal standard, with peaks at 0 ppm for ^1H and −2.8 ppm for ^{13}C. The conditions for ^1H NMR were a 40° pulse angle, a 30 s delay between pulses, a 6.0 kHz spectral width, 32K data points, and 32 scans. The conditions for ^{13}C NMR were a 60° pulse angle, a 3 s delay between pulses, a 23.0 kHz spectral width, 32K data points, and 20 000 scans. A proton-decoupled ^{13}C NMR spectrum without a nuclear Overhauser effect was measured with a 30 s delay, and it was used for quantification.

The DQF−COSY[13] data were acquired in the magnitude mode using 80 scans for each of the 256 t_1 increments, a 2.0 s recovery delay, spectral widths in f_1 and f_2 of 2.7 kHz, an acquisition time of 0.379 s, and 12.0 μs 90° ^1H pulses. Data were processed with shifted sine bell weightings and zero-filled to a 1K × 512 data matrix.

The $^{13}C-^1H$ heteronuclear correlation (HETCOR[14]) spectrum was acquired in the magnitude mode using 112 scans for each of the 256 t_1 increments, a 1.4 s recovery delay, spectral widths in f_1 and f_2 of 2.7 and 4.0 kHz, respectively,

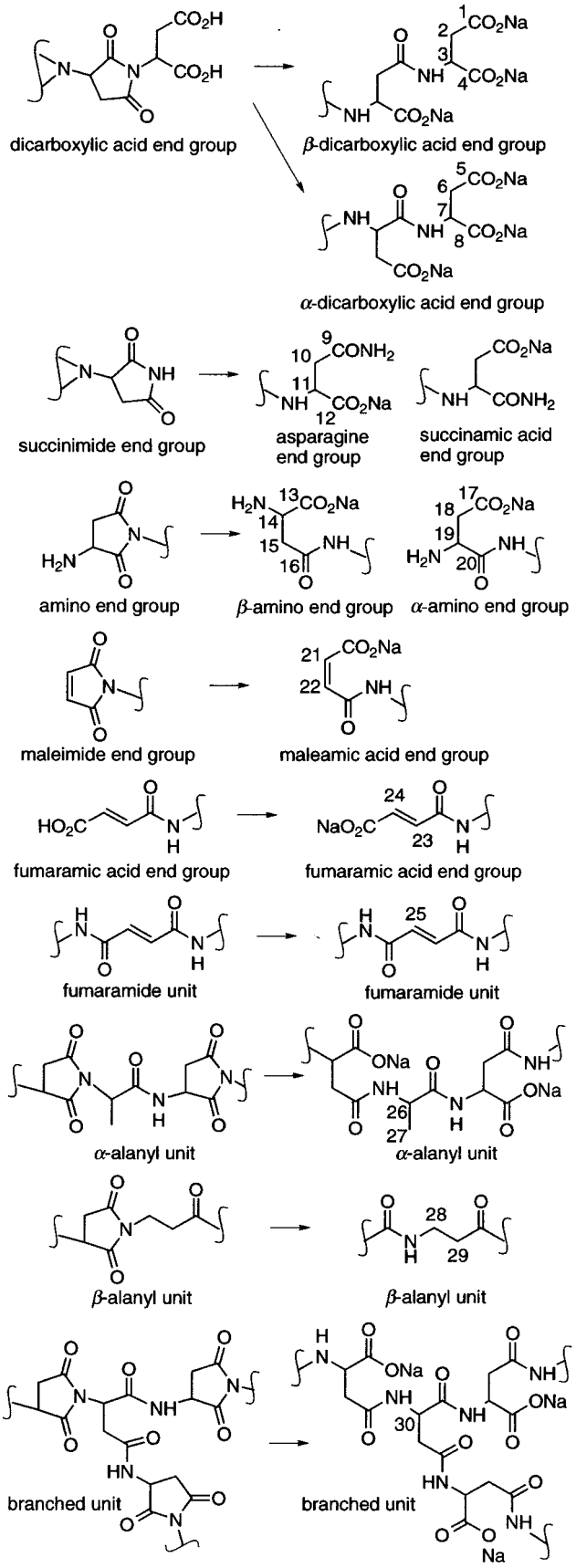


Figure 1. End groups and irregular structures of poly(succinimide) and the corresponding structures of sodium polyaspartate after hydrolysis. Numbering of each nucleus is indicated for the NMR assignment of sodium polyaspartate.

an evolution delay (1/(2*J*)) of 3.8 ms, an acquisition time of 0.256 s, 26.0 μs 90° ^{1}H pulses, and 9.5 μs 90° ^{13}C pulses. Data were processed with exponential weightings multiplied by a trapezoidal function and zero-filled to a 1K × 512 data matrix. The ^{13}C−^{1}H correlation via long-range couplings (COLOC[15]) 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 2.7 and 22.0 kHz, respectively, a long-range evolution delay (1/(2J)) of 50 ms, an acquisition time of 0.093 s, 26.0 μs 90° ^{1}H pulses, and 9.5 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.

Table 2. ^{1}H and ^{13}C NMR Chemical Shifts of End Groups and Irregular Structures of Thermally Prepared Sodium Polyaspartate[a]

	chemical shift (ppm)			chemical shift (ppm)	
assignt[b]	^{13}C	^{1}H	assignt[b]	^{13}C	^{1}H
1	179.0		16	171.1	
2	39.8	2.52, 2.7	17	176.4	
3	53.2	4.43	18	37.5	2.69, 2.83
4	178.8		19	51.0	4.22
5	179.0		20	170.2	
6	39.6	2.54, 2.65	21	*c*	5.94[d]
7	53.1	4.37	22	*c*	6.40[d]
8	178.3		23	*c*	6.72[e]
9	175.8		24	*c*	6.67[e]
10	*c*	*c*	25	*c*	6.92[d]
11	*c*	4.58[d]	26	*c*	4.17
12	177.4		27	*c*	1.34
13	174.0		28	*c*	3.38
14	51.4	4.01	29	*c*	2.38
15	35.3	2.87, 2.98	30	*c*	4.75[d]

[a] NMR spectra were obtained at 24 °C and pD 8 in D_2O solution unless otherwise mentioned. [b] Numbering of each nucleus is shown in Figure 1. [c] Not assigned due to the low concentration or the overlapping of signals. [d] Measured at 60 °C. [e] Assignments may be interchanged.

Results and Discussion

Using the knowledge of the end groups and irregular structures of PSI,[10] we can predict those of SPA, which is synthesized by the hydrolysis of PSI. Because the M_w of SPA is not much lower than that of the original PSI, we can approximately assume that the imide linkages are hydrolyzed to the α- and β-amide linkages under the hydrolysis conditions employed here while the amide linkages are left intact as shown in Figure 1. The terminal residues of the dicarboxylic acid end group and the fumaramic acid end group and the central residues of the fumaramide unit, the α- and β-alanyl units, and the branched unit in PSI would remain unchanged, while the imide linkages in the adjacent residues would be hydrolyzed to the amide linkages. The succinimide, amino, and maleimide end groups in PSI would become the corresponding end groups with acyclic amide linkages.

The ^{1}H and ^{13}C NMR spectra of SPA-1 are shown in Figures 2a and 3b, respectively, and the assignments of the end groups and irregular structures are summarized in Table 2. The numbering of each nucleus of SPA is also shown in Figure 1. The assignments of the signals are obtained for the most part by a combination of the following: (1) analysis of the proton−proton and proton−carbon connectivity in the 2D NMR spectra; (2) internal consistency in the ^{1}H and ^{13}C NMR signal areas; (3) comparison of the chemical shifts of SPA-1 with those of the model compounds; (4) the pD dependence of the ^{1}H and ^{13}C chemical shifts.

Partial or complete racemization is expected to occur during the thermal synthesis of PSI.[6] As a result, the

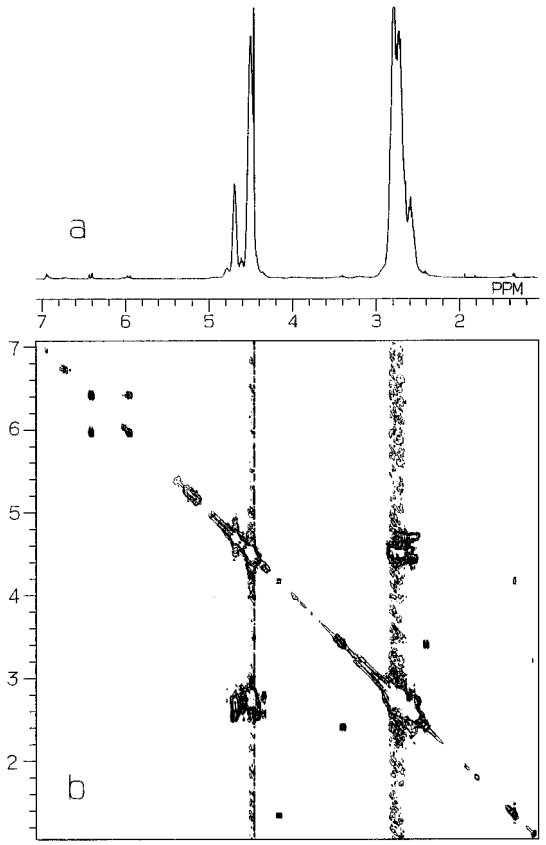


Figure 2. (a) ^{1}H and (b) DQF−COSY NMR spectra of thermally prepared sodium polyaspartate measured at 60 °C in D_2O.

SPA prepared from the racemized PSI should show the split NMR signals due to the difference in stereoregularity. Little splitting is caused by racemization, however, probably because the two adjacent methine carbons are separated by three or four chemical bonds in SPA.

Structure of the Main Chain. The proton signals at 4.66 and 4.47 ppm have already been assigned to the methine protons with the α- and β-amide units, respectively,[3] using the SPA prepared by debenzylation of poly(β-benzyl aspartate) and connected with only an α-amide bond. The ratio of α- to β-amide units in SPA-1 is 28 to 72, and this ratio is comparable to those reported in the literature.[5,8,16] Corresponding methylene protons can be assigned to the signals in the regions of 2.48−2.73 and 2.59−2.82 ppm, respectively, using the DQF−COSY spectrum shown in Figure 2b. The methine carbons with the α- and β-amide units are assigned to the signals at 51.4 and 51.9 ppm, respectively, using the HETCOR spectrum shown in Figure 4. The methylene carbons with the α- and β-amide units are assigned to the signals at 39.1 and 37.7 ppm, respectively, in a similar way. The ^{13}C NMR signals of the three amide regions at 173.3, 172.7, and 172.0 ppm have been assigned to the two-unit sequences αα, αβ + βα, and ββ from high to low frequency by Pivcová et al.[5] on the basis of the population change with the ratio of α- to β-amide units, and the assignments have been confirmed by Wolk et al.[8] using the ^{1}H−^{13}C HMBC spectrum. The carboxylate carbons can be assigned to the signals in the 177−178 ppm region.

Structures of End Groups. Figure 3 also shows the ^{13}C NMR spectra of the model polymers. Comparison of the spectrum of high molecular weight SPA-2

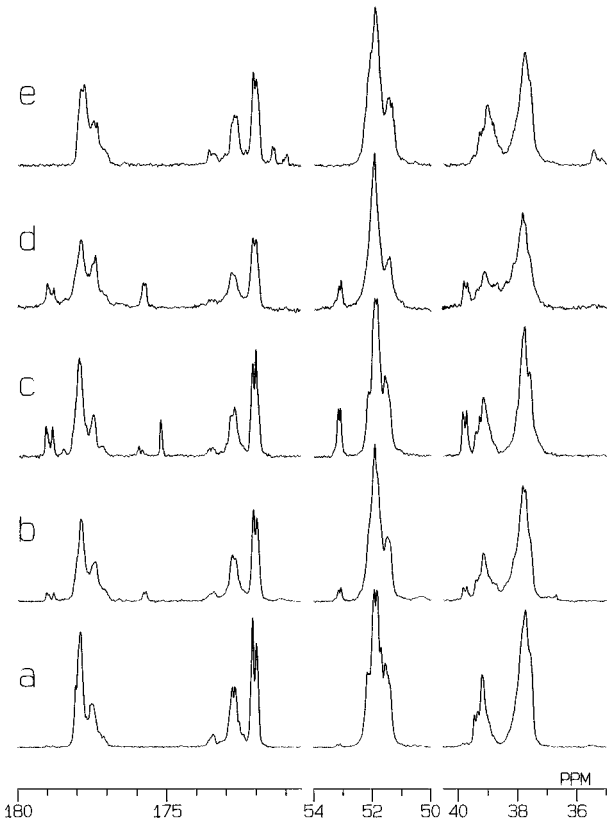


Figure 3. ^{13}C NMR spectra of sodium polyaspartate and model polymers measured at 24 °C and pD 8 in D_2O: (a) high molecular weight SPA-2; (b) SPA-1 thermally prepared without phosphoric acid catalyst; (c) SPA-4 with many dicarboxylic acid end groups; (d) SPA-3 with many asparagine end groups; (e) SPA-5 with many amino end groups.

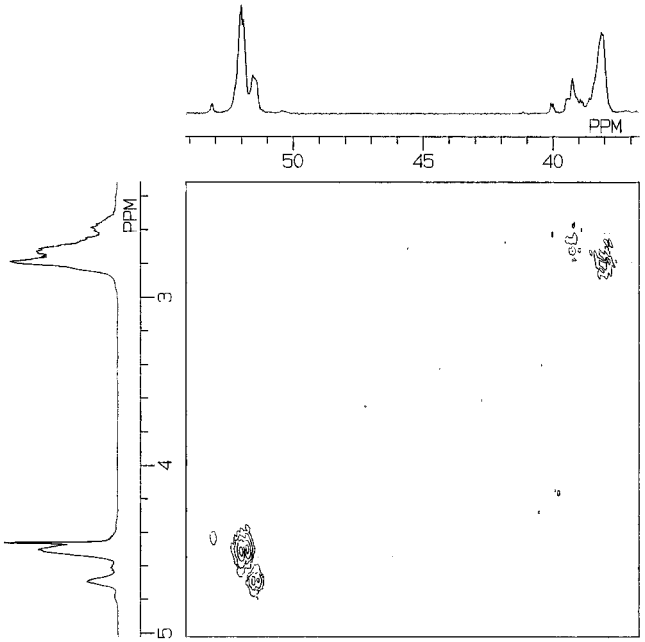


Figure 4. ^{13}C−^{1}H HETCOR spectrum of thermally prepared sodium polyaspartate measured at 60 °C in D_2O.

with that of SPA-4 possessing many dicarboxylic acid end groups suggests which signals are ascribed to the α- and β-dicarboxylic acid end groups. The oligomer of sodium aspartate connected with only a β-amide bond was utilized to assign C-1 to C-4 of the β-dicarboxylic acid end group as shown in Table 2. Asp-Asp-Asp,

which is a model compound of the α-dicarboxylic acid end group, was similarly utilized to assign C-5 to C-8 carbons. C-1 and C-5 can be distinguished from C-4 and C-8, respectively, from the coincidence of the chemical shifts of C-1 and C-5 and the observation that most of the two-bond correlation signals are stronger than the three-bond signals in the COLOC spectrum. The methine and methylene protons are assigned using the HETCOR spectra of the model compounds as shown in Table 2. The signals ascribed to the α- and *β*-dicarboxylic acid end groups are not observed in the spectrum of SPA-5 (Figure 3e) in which the C-terminal is end-capped with 3,4-dimethylaniline and the α- and *β*-dicarboxylic acid end groups are not present. This comparison can also be used to confirm the assignment of the structure.

The signals at 175.0 and 175.8 ppm in the ^{13}C NMR spectrum of SPA-4 (Figure 3c) are ascribed to the amide carbonyl carbons in the succinic acid unit used as an end-capping agent. The carboxylate carbons in the succinic acid unit resonate at 181.3 and 181.2 ppm. Two sets of signals are probably observed due to the effect of whether the penultimate aspartyl unit is connected with an α- or *β*-amide bond. The methylene carbons in the succinic acid unit resonate at 32.0 and 32.7 ppm; the former is connected to the amide carbonyl carbon, and the latter is connected to the carboxylate carbon. The carbon signals in the succinic acid unit are assigned using the pD change. When the pD of the solution is decreased from high to low, the carboxylate carbon is acidified to the carboxylic acid and its chemical shift is shifted toward a low frequency, while that of the amide carbonyl carbon is less pD-dependent. The methylene carbon connected to the carboxylate carbon can be assumed to shift more than the other.[4]

Comparison of the ^{13}C NMR spectrum of SPA-2 with that of SPA-5 possessing many amino end groups suggests which signals are ascribed to the α- and *β*-amino end groups. The oligomer of sodium aspartate connected with only a *β*-amide bond was utilized to assign the C-13 to C-16 of the *β*-amino end group. Asp-Asp-Asp, which is a model compound of the α-amino end group, was utilized to assign C-17 to C-20 carbons. The methine and methylene protons are assigned using the HETCOR spectrum as shown in Table 2.

PSI-3 with many succinimide end groups and dicarboxylic acid end groups can be prepared from ammonia and maleic anhydride. The hydrolysis of the PSI should produce the SPA with many asparagine end groups and α- and *β*-dicarboxylic acid end groups. From the comparison of the ^{13}C NMR spectrum of SPA-1 with that of SPA-3 and from the pD change described before, we can assign C-9 of the asparagine end group to the signal at 175.8 ppm and C-12 to that at 177.4 ppm. The chemical shifts of DL-alanyl-L-asparagine, 175.8 and 177.0 ppm, respectively (the difference in the chemical shifts for each carbon between the two isomers are within 0.2 ppm), agree well with those of the end group. The methine and methylene carbons in the asparagine end group are overlapped with the main chain signals. A succinamic acid end group was not identified, probably due to its low concentration.

The olefinic proton signals at 5.94 and 6.40 ppm are coupled by 12.8 Hz. We can assign these signals to the maleamic acid end group, because the olefinic protons of *N*-cyclohexylmaleamic acid show almost the same chemical shifts and coupling constant as shown in Figure 5. The lower the pD becomes, the more the signal at 5.94 shifts toward a high frequency, while the signal at 6.40 ppm scarcely shifts. Therefore, the signals at 5.94 and 6.40 are assigned to H-21 and H-22, respectively.

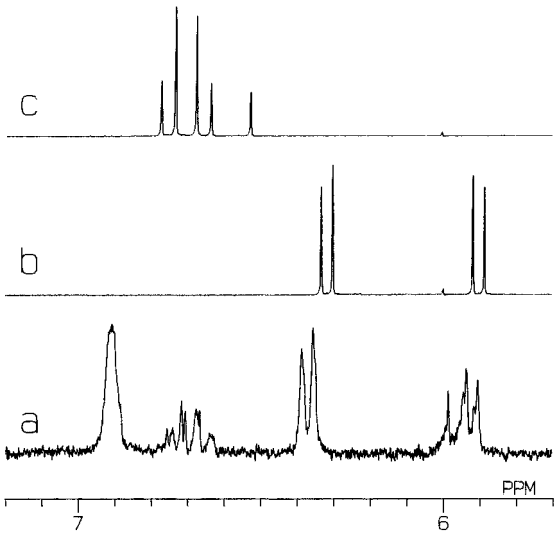


Figure 5. Olefinic proton region of the ^{1}H NMR spectra of (a) sodium polyaspartate thermally prepared without phosphoric acid catalyst, (b) *N*-cyclohexylmaleamic acid, and (c) fumaramic acid.

The fumaramic acid end group is not expected to change through hydrolysis. The olefinic proton signals at 6.67 and 6.72 ppm are coupled by 15.9 Hz. We can assign these signals to the fumaramic acid end group, because the olefinic protons of fumaramic acid show almost the same chemical shifts and coupling constant as shown in Figure 5. The signals at 5.99 and 6.52 ppm are olefinic protons of maleic acid and fumaric acid, respectively.

Irregular Structures in the Main Chain. The proton signal at 6.92 ppm is pD-independent, and the olefinic proton of the fumaramide unit in PSI resonates at 6.81 ppm in DMSO-d_6.[10] Accordingly, we can assign the signal to H-25 of the fumaramide unit in SPA. A maleamide unit with a cis configuration should resonate at a lower frequency.

The doublet proton signal at 1.34 ppm is coupled to the quartet at 4.17 ppm by 7.3 Hz in the DQF−COSY spectrum. These signals are assigned to the α-alanyl unit, which is most likely produced by decarboxylation of the *β*-carboxylic acid of the aspartyl residue during polycondensation.

The triplet proton signal at 3.38 ppm is coupled to the triplet at 2.38 ppm by 7.3 Hz in the DQF−COSY spectrum. These signals can be ascribed to the *β*-alanyl unit most likely produced by the decarboxylation of the α-carboxylic acid.

The methine proton region of SPA-1 was closely investigated to confirm the presence of the branched unit. Figure 6 shows the ^{1}H NMR spectra of SPA-1 and the model polymers measured at 80 °C. At this temperature, a residual water signal resonates at 4.25 ppm and does not disturb the interpretation of the spectrum. The methine protons of both the α- and *β*-dicarboxylic acid end groups resonate at 4.4 ppm (Figure 6d). The methine proton of the asparagine end group resonates at 4.58 ppm (Figure 6e). The methine proton of the aspartyl residue with a laurylamide unit in SPA-6 resonates at 4.75 ppm (Figure 6c). The comparison of

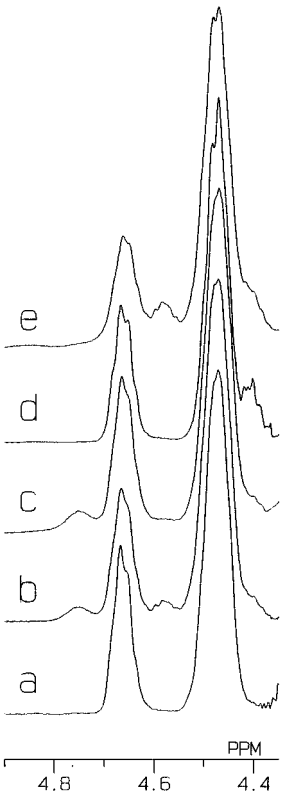


Figure 6. ^{1}H NMR spectra of sodium polyaspartate and model polymers measured at 80 °C and pD 8 in D_2O: (a) high molecular weight SPA-2; (b) SPA-1 thermally prepared without phosphoric acid catalyst; (c) SPA-6 with many laurylamide branched units; (d) SPA-4 with many α and β-dicarboxylic acid end groups; (e) SPA-3 with many asparagine end groups.

the signal area of the methine proton at 4.75 ppm with that of the methylene proton next to the nitrogen atom in the laurylamide reveals that both methine protons in the α- and β-laurylamide units resonate at 4.75 ppm. The signal at 4.75 ppm in the spectrum of SPA-1 (Figure 6b) can be assigned to the methine proton of the branched unit. Whether the adjacent residue is connected with an α- or β-amide bond has little influence on the methine chemical shift in the branched unit. The effect of the amide sequence and the stereoregularity on the line width seems to be at most the size observed in the spectrum of high molecular weight SPA-2 (Figure 6a).

Using a model SPA with 15% unhydrolyzed succinimide units, Wolk et al.[8] demonstrated that the proton signals of the residual succinimide units resonate at 4.9, 3.1, and 2.75 ppm. These signals were not clearly observed under the present experimental conditions because the succinimide is stable in acidic aqueous solution but reacts with water in neutral or basic medium.[16]

Quantity of Minor Structures in SPA-1. The end groups and irregular structures of SPA are quantified on the basis of the above-described assignments. The methylene proton signals in the region of 2.3−3.1 ppm and the carbonyl carbon signals in the region of 171−179 ppm are used to estimate the total monomer units in the main chain. The proton and carbon signals in the following parentheses are used to estimate the quantity of each minor structure. The calculated quantities of the end groups and irregular structures of SPA-1 per 100 monomer units are as follows: the α- + β-amino end groups (H-19 and H-14), 0.3; the maleamic acid end group (H-22), 0.6; the fumaramic acid end group (H-23 and H-24), 0.2; the fumaramide unit (H-25), 0.4; the α-alanyl unit (H-27), 0.3; the β-alanyl unit (H-28), 0.1; the branched unit (H-30), 2.9; the α-dicarboxylic acid end group (C-8), 0.8; the β-dicarboxylic acid end group (C-4), 2.8; the asparagine end group (C-9), 5.2.

The α- and β-amino, maleamic acid, and fumaramic acid end groups are N-termini in the polymer. The fumaramide unit is also considered as two coupled N-termini. The branched unit corresponds to an N-terminus. On the other hand, the α- and β-dicarboxylic acid end groups and the asparagine end group are C-termini. If the major end groups and irregular structures are assumed to be assigned here, the quantitative relation of the minor structures is expressed by the following equation:

$$N_{ae} + N_{me} + N_{fe} + 2N_{fu} + N_{bu} = N_{de} + N_{age}$$

N_{ae} is the quantity of the α- + β-amino end groups per 100 monomer units, N_{me} is that of the maleamic acid end group, N_{fe} is that of the fumaramic acid end group, N_{fu} is that of the fumaramide unit, N_{bu} is that of the branched unit, N_{de} is that of the α- + β-dicarboxylic acid end groups, and N_{age} is that of the asparagine end group. The sum of the left side of the equation is 4.8 and that of the right side is 8.8 for SPA-1. The sum of the left side is less than that of the right side. This discrepancy is probably caused in part by unidentified minor structures possessing units which invert the head-to-tail sequence of the monomer unit, such as a diketopiperazine unit and an iminodisuccinic acid unit. An error in the integration of partially overlapped signals is likely another, less significant, reason. The separation of the methine proton region was not much improved even in the spectrum measured by a 600 MHz NMR spectrometer probably because the dispersion in the chemical shift significantly affects the line width; thus the higher the observation frequency, the broader the line width becomes.

The average number of monomer units between two adjacent branch points and between a branch point and an end group in SPA can be estimated from the quantity of the branched units using an expression, $50M_n/(N_{bu}M_n + 50M_a)$, where M_n is the number-average molecular weight and M_a is the molar mass of the repeating aspartyl units in the polymer. The ring structure is neglected in this expression. The estimated value for SPA-1 is 13 when the M_n measured by GPC is employed to calculate it.

Relation of PSI to SPA. The quantities of the minor structures of SPA-1 are expected to have a correlation with those of the original PSI-1. The quantities of the end groups and irregular structures of PSI-1 per 100 monomer units are as follows:[10] the maleimide end group, 1.0; the fumaramic acid end group, 0.3; the fumaramide unit, 0.4; the α-alanyl unit, 0.2; the dicarboxylic acid end group, 4.1; the succinimide end group, 6.5. The comparison of these quantities with those of the corresponding structures of SPA-1 leads us to the conclusion that side reactions scarcely occur during the hydrolysis of PSI to SPA under the present experimental conditions; that is, the succinimide ring is hydrolyzed to the α- and β-amide units while the amide bond is basically not hydrolyzed. In contrast, side reactions occur during the synthesis of PSI by the polycondensation of aspartic acid, and the production

of most of the irregular structures can be ascribed to the side reactions such as deamination, decarboxylation, and chain scission.[10]

The quantity of the branched units could not be determined in the analysis of PSI because the amide proton signals of the branched unit and unclosed amide unit as well as other ^{1}H and ^{13}C signals are severely overlapped. Because the amount of the branched unit of SPA is determined in this experiment, the amount of the unclosed amide unit in the PSI can be determined by subtraction if it is confirmed that the branched unit is not hydrolyzed to an amino end group and a linear chain. It is also expected that structure−property relationships can be investigated more accurately than the results in which the total amount of the amide protons in PSI is used as an indicator of the amount of the branched units in SPA.[9]

Conclusions

The combination of the detailed knowledge of the end groups and irregular structures in PSI and the specially prepared model compounds made it possible to identify and quantify the end groups and irregular structures in thermally prepared SPA. However, the sum of the C-termini and that of the N-termini did not coincide; this suggests the presence of unidentified irregular structures which invert the head-to-tail sequence of the monomer unit. The comparison of the quantities of the minor structures in SPA with those of the corresponding ones in PSI revealed that side reactions scarcely occur during the hydrolysis of PSI to SPA.

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