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In situ polymerization of starch with lactic acid in aqueous solution and the microstructure characterization

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Abstract

Copolymers of starch grafted with poly (lactic acid) were directly prepared by in situ reaction of cornstarch with lactic acid in aqueous media in the presence of stannous 2-ethyl hexanoate [$Sn(Oct)_2$]. The procedure of the graft reaction was elucidated, based on the HPLC analysis, as the ring-opening polymerization (ROP) from starch surface of the small amounts of lactide produced in situ in the reaction system. The detailed chemical microstructure of the resultant graft copolymer was clarified by one- and two-dimensional NMR spectroscopy. Analysis of ^{13}C NMR spectra of the carbons in starch ring as well as the carbonyl carbons in LA moiety demonstrates that the reactivity of hydroxyl groups at glucopyranan unit of starch decreases in the order of C-6 > C-3 > C-2. The average length of PLA grafts at individual starch ring carbons was also investigated.

Keywords: Starch; Poly(lactic acid); Relative reactivity; Degree of substitution; Degree of polymerization; ^{13}C magnetic resonance

1. Introduction

Starch, the generic name for the D-glucose storage polysaccharide in plants, consists of a mixture of two polysaccharides amylose and amylopectin (Van der Burgt et al., 2000). Amylose is mainly a linear (1→4)-α-D-glucopyranan units and with a limited degree of (1→6)-α-D branching. Amylopectin, highly ordered semi-crystalline, comprises (1→4)-α-D-glucopyranan units with (1→6)-α-D linkages. Extensive studies have been stressed on the application of starch as biodegradable plastics for its nontoxic, natural abundance and low cost. However, like most polysaccharides, the high hydrophilicity, poor processability and solubility in common organic solvents limit wide applications of starch. Therefore, the modification of starch, physically and chemically, has been extensively studied.

Chemical modifications of starch, including chemical derivation and graft copolymerization, are efficacious methods to improve the properties of starch. Lots of studies have been reported in literatures about the chemical derivation of starch. Fang, Fowler, Sayers, and Williams (2004) modified a range of starches in aqueous sodium hydroxide (NaOH) solution with acid chlorides. Shogren (2003) prepared a series of starch esters, such as starch acetates and starch succinates by high temperature and pressure reaction. Starch esters with moderate to high degree of substitution (DS) may find applications as biodegradable packaging materials (Fang et al., 2004; Shogren, 2003; Aburto, Alric, Borredon, & Cedex, 1999).

Graft copolymerization, as a rational approach of chemical modification, has been extensively used in the modification of starch. Starch graft copolymers, such as starch-g-poly(acrylic acid)(PAA) (Athawale and Lele, 1998) and starch-g-polystyrene (PS) (Cho & Lee, 2002) were prepared by free radical copolymerization. In fact, owing to the environmental and ecological concerns, researches on biodegradable compositions based on degradable polyester grafted starch-like polysaccharides have been focused on for decades. Grafting the synthetic degradable polyesters onto starch not only offers an effective way of improving the physical properties of starch, but also imparts thermoplastic properties to starch producing fully biodegradable materials. Isocyanates, such as 2,4-tolylene diisocyanate (TDI), methylenediphenyl diisocyanate (MDI), were investigated as chain extender in graft copolymerization of starch with biodegradable polyesters, like polycaprolactone (PCL) (Mani, Tang, & Battacharya, 1998), poly (lactic acid) (PLA) (Wang, Sun, & Seib, 2002). However, isocyanates are considered environmentally hazardous materials. Accordingly, Zhang and Sun (2004) reported grafting of starch with maleic

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anhydride onto PLA. A series of studies have been bulletined concerning on the controlled graft copolymerization of L-lactide onto starch-like polysaccharides, such as pullulan and dextran in tetrahydrofuran (THF) solution (Ohya, Maruhashi, & Ouchi, 1998; Ouchi, Kontani, & Ohya, 2003; Teramoto & Nishio, 2003; Donabedian & McCarthy, 1998). Recently, Dubois, Krishnan, and Narayan (1999) reported on the in situ ring-opening polymerization (ROP) of ε-caprolactone in the presence of starch in bulk (without solvents). Choi, Kim, and Park (1999) explored the preparation of starch-g-polycaprolactone copolymers by the ROP of caprolactone monomer. In their reaction system, water was used as swelling agent.

In this paper, we found that starch-g-PLA copolymer was formed when cornstarch was directly subjected to lactic acid. The graft reaction was conducted in an aqueous media in the presence of $Sn(Oct)_2$. Despite the extensive studies on the modification of starch so far, we found that some confusions in the assignments of ^{13}C-shifts of starches and their derivatives remained unclarified (Dicke, Rahn, Haack, & Heinze, 2001; Choi et al., 1999; Heins, Kulicke, Käuper, & Thielking, 1998). Therefore, the chemical microstructure of the resultant graft copolymer was clarified by one- and two-dimensional NMR study. Based on the specific assignment of the ^{13}C NMR signals of starch grafts, additional attempts were made to establish the actual position of lactyl units at the glycopyranan ring. Furthermore, the DS of LA grafted onto starch as well as the average length of PLA grafts (the degree of polymerization, DP) was demonstrated. This free-organic solvent reaction process gives an economical, facile and 'environmentally friendly' method for the preparation of starch grafted PLA copolymers.

2. Experimental section

2.1. Materials

Cornstarch (27.7% amylose, Maoyuan Starch Co., China) and aqueous L-lactic acid (containing 20 wt% water) (Acros Organics, Belgium) were chemical grade and used without further purification. The PLA used for FTIR and NMR analysis was 'H-100' purchased from Mitsui Chemicals, Japan.

All other chemicals were analytical grade and used as received.

2.2. In situ reaction of lactic acid with starch

Cornstarch (10 g) and the aqueous lactic acid solution (112.5 g, net content of LA was 90 g) were added to a 250 mL of three-necked flask equipped with a mechanical stirrer and vacuum pump system. The temperature of the reaction system was thermostated by a temperature controlling system. Starch was first gelatinized at 75 °C. When most of water was drawn out, $Sn(Oct)_2$ (0.3 wt% of total amount of the reactants, exclude the water amount) was added to the flask. The graft copolymerization was proceeded at 90 °C under vacuum (<1 mm Hg) for predetermined time. At the completion of reaction, the system was cooled to room temperature. The resultant product was washed twice with acetone under vigorous stirring. Then the product was further purified by Soxhlet extraction to remove completely the unreacted LA monomer as well as the PLA homopolymer that may be formed during the graft reaction. The extraction was performed for 72 h with acetone as the extracting solvent. The final product was dried at 60 °C under vacuum.

To confirm the in situ formation of lactide, a blank reaction without cornstarch was manipulated by the same procedure. After reacting for predetermined time, part of the reaction product (0.3 g) was taken out of the reaction system. The product was dissolved in acetonitrile (HPLC grade) and used for HPLC analysis. The reaction was performed for 5, 7, 9 and 11 h, respectively.

2.3. Measurements

FTIR Analysis. Fourier transform infrared (FTIR) spectra were recorded on a Bruker vector 22 instrument (Bruker, Germany). The starch graft copolymers were dissolved in dimethyl sulfoxide (DMSO) and PLA was dissolved in chloroform. The solution was cast on NaCl plates. The solvent was completely removed under vacuum.

HPLC analysis. The characterization of lactide by HPLC spectroscopy was carried out on a LC-10AD HPLC system (Shimadzu, Japan) equipped with a binary bump (LC_10AD VP), a reversed-phase column (Diamosil^{TM} C_{18}, 5-μm pore size, 250×4.6 mm) and a UV–visible absorption detector (SPD-10AVP) (Shimadzu, Japan). A mixture of acetonitrile and water (in 30:70 v/v) was used as the eluent and the flow rate was of 1.0 mL/min. The elution was monitored by UV absorption at 254 nm. Lactide contained in the samples was identified by comparing the retention time of the relevant peak of the samples with that of the corresponding standard specimen. External standard chromatographic method was adopted in quantification.

NMR Analysis. NMR spectra were obtained on a Bruker AV400 spectrometer (Bruker, Germany) at 400 MHz for ^1H-NMR and 100 MHz for ^{13}C NMR, respectively. One- and two-dimensional NMR measurements were conducted to obtain the detailed information on the microstructure of starch-g-PLA copolymer, including the DS and DP. Starch as well as the graft copolymer was dissolved in DMSO-d6 where the concentration was about 15 (w/v)%. The analysis for starch copolymers was carried out at 60 °C. PLA was dissolved in chloroform-d1. For ^1H-NMR, the spectra were obtained with a pulse angle of 25°, the delay time of 2 s and an acquisition time of 10 s. All of the chemical shifts were reported in parts per million (ppm) downfield from 0.00 ppm using tetramethylsilane (TMS) as the internal reference. Residual undeuterated protons in DMSO-d6 were set at 2.50 ppm. For quantitative ^{13}C NMR, DMSO was a reference line with a septet centered at 40.0 ppm. Two-dimensional heteronuclear multiple quantum coherence (HMQC) spectra were carried out to obtain detailed information on the connectivity of each carbon and its directly

attached proton in the graft copolymer. The analysis of HMQC spectra enables us to conduct the assignment of the characteristic resonance and to elucidate the fine chemical microstructure of starch graft copolymer.

3. Results and discussion

3.1. Synthesis of starch-g-PLA copolymer

In situ polymerization of starch with lactic acid in aqueous solution was conducted using $Sn(Oct)_2$ as catalyst. The first step of synthesis of PLA-grafted starch consists of the gelatinization of starch in aqueous lactic acid solution at 75 °C. The starch gelatinization generally induces the effective destructurisation of the semi-crystalline starch granules and plasticization of granular structure of starch and, consequently, results in a homogeneous reaction system to promote the modification of starch. The second step depicted in Fig. 1 consists of the graft copolymerization of LA and starch. It is known that the reactivity of the hydroxyl group in starch with carboxyl group is generally undetectable, especially under acidic condition. This conclusion is further proved by one of our attempt to react starch with proionic acid where the same reaction condition as described above was employed. As expected, no esterificated product was found. The proposed chemical reactions among lactic acid and starch are shown in Fig. 1A. In order to validate the proposed copolymerization, HPLC analysis was conducted. The result shows that small amounts of lactide were included in the reaction system (Fig. 2A). It is believed that lactide was formed in situ in the reaction system since no lactide can be detected in the original aqueous lactic acid solution. The result also demonstrates that the amount of lactide inside the reacting system increased with the prolongation of the reacting duration (Fig. 2B). Accordingly, it gives rise to the suggestion that the graft copolymerization of starch with lactic acid in aqueous solution is probably through the ROP of lactide (Fig. 1A). The ROP of lactide from starch surface results in the attachment of LA to starch backbone via transesterification of lactide to starch. The initiation of the ROP of lactide is suggested to proceed from hydroxyl groups of the starch phase catalyzed by $Sn(Oct)_2$ (Kricheldorf, Sauders, & Stricker, 2000; Korhonen, Helminen, & Seppälä, 2001). The ^{13}C NMR spectrum of the starch-*g*-PLA sample (Fig. 6B) shows an additional signal at 173 ppm indicating the carbonyl group of LA near the end hydroxyl group (Hiltunen, HärkÖnen, Seppälä, & Väänänen, 1996). Therefore, the ring-opening polymerization should occur through acyl-oxygen cleavage.

3.2. The assignment of the characteristic resonance in starch-g-PLA copolymer

FTIR spectrum shows the characteristic absorption of PLA carbonyl stretching at 1749 cm^{-1} (Fig. 3). This strong absorption indicates that the graft copolymer of starch with PLA is resulted in the reaction system.

The microstructure of starch-g-PLA was further elucidated by means of NMR spectroscopy. Numbers 1–6 are designed to indicate the carbons at the glucopyranan unit of starch and the symbols of a to c represent the carbons in PLA moiety. The similarity of chemical shift parameters between acetylated starch and lactylated starch enables the determination of the position of lactyl units at the glucopyranan ring and the overall distribution.

The ^1H-NMR spectra of cornstarch, PLA and starch-g-PLA are shown in Fig. 4. Based on the reports on the peak assignment for proton species in amylose, amylopectin, starch and starch derivatives with similar environment to starch-g-PLA (Peng & Perlin, 1987; Gagnaire, Mancier, & Vincendon, 1978; Nilsson, Bergquist, Nilsson, & Gorton, 1996; Friebolin, Keilich, & Siefert, 1969; Largnel, Bliard, Massiot, & Nuzillard, 1997), we assigned the proton signals in the NMR

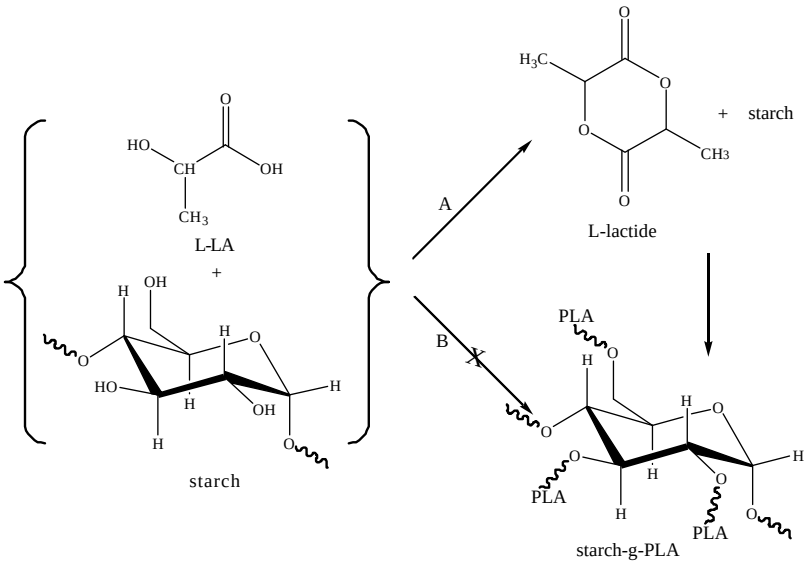

Fig. 1. Proposed reaction between starch and lactic acid.

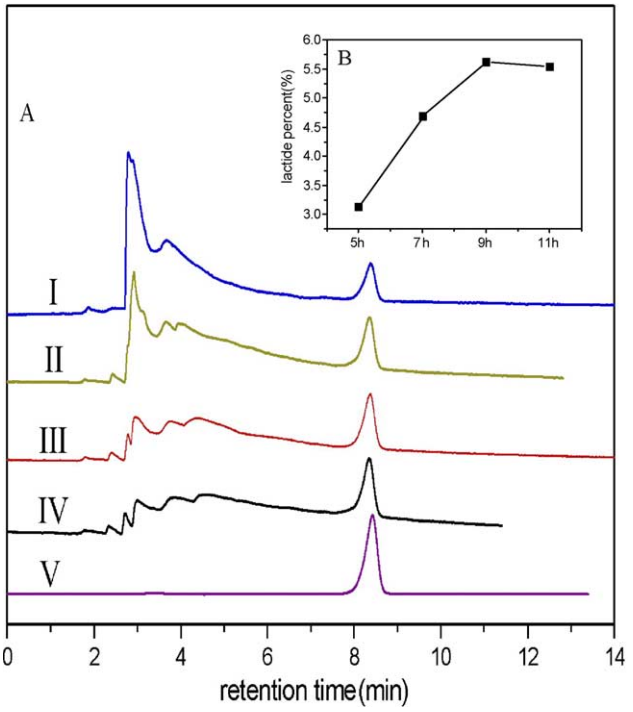

Fig. 2. HPLC spectra of reaction mixures with different reaction time (A), and the concentration of lactide with the prolongation of reaction time (B). I-IV means the samples with the reaction time of 5h, 7h, 9h and 11h, respectively. V indicates the standard sample of L-lactide.

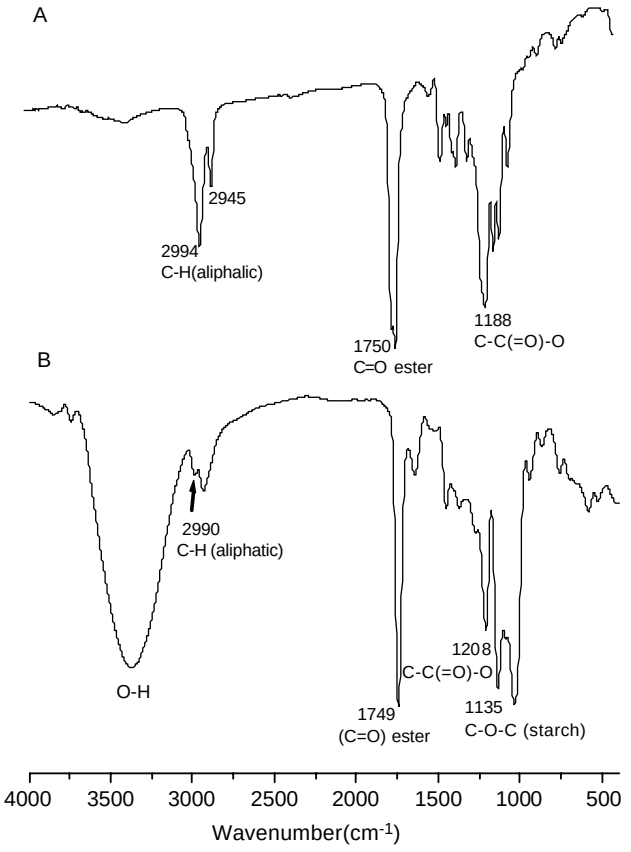

Fig. 3. FTIR spectra of PLA (A), and starch-g-PLA (B).

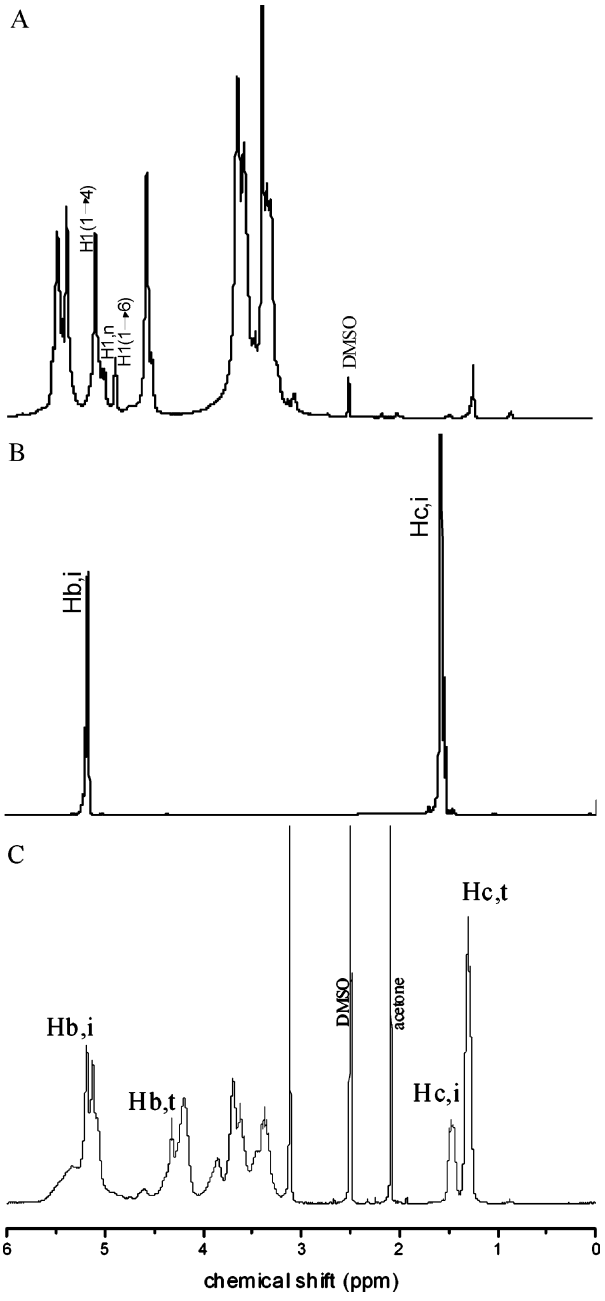

Fig. 4. ^{1}H-NMR spectra of cornstarch (A), PLA (B) and starch-g-PLA (C).

spectrum of starch-g-PLA as quoted in Table 1. The degree of amylopectin branch can be calculated from the ^{1}H-NMR spectrum of cornstarch (Fig. 4A) as follows:

$$\mathrm{DB} = \frac{I(1,b)}{[I(1,b) + I(1,l) + I(1,n)]}$$

where I(1,b), I(1,l) and I(1,n) are the integrals of the anomeric proton at the branch point of amylopectin, the anomeric proton at starch ring with a linear linkage and the anomeric proton at the nonreducing end of amylopectin, respectively. For our system, the DB value is 0.16, implying that there are 16 amylopectin branches per 100 glucopyranan units in the cornstarch used in this paper.

Table 1
Chemical shift assignments for ^{13}C- and ^{1}H- species of starch-g-PLA

^{13}C assignment	Chemical shift (ppm)	^{1}H assignment	Chemical shift (ppm)
C1,n[a]	100.4	H1,n	5.02
C1[b]	99.9	H1(1–4)	5.09
C1-s2[c]	95.7	H1(1–6)	4.90
C2	71.9	H1-s2	5.35
C2s[d]	70.6	H2	3.36
C2-s3	69.5	H2s	4.60
C3	73.1	OH-2	5.39
C3s	71.6	H3	3.62
C3-s2	70.5	H3s	5.45
C4	78.8	OH-3	5.49
C4,n	70.3	H4	3.38
C4-s3	72.6	H4-s3	3.70
C5	71.5	H5	3.60
C5,n	72.6		
C5-s6	68.6	H5-s6	3.86
C6	60.4	H6	3.69-3.60
C6,n	60.6	H6s	4.20
C6s	62.8	OH-6	4.60
Ca,t[e]	174.2,173.9,173.7		
Ca,i[f]	169.8,169.4,169.0		
Cb,t	65.8–65.6	Hb,t	4.34–4.31
Cb,i	67.9–67.7	Hb,i	5.19
Cc,t	20.1	Hc,t	1.30–1.28
Cc,i	16.5	Hc,i	1.49–1.45

[a] C1,n indicates the C-1 carbon at a non-reducing end of starch.
[b] C1 donates the C-1 carbon at the main residue of starch.
[c] C1-s2 means the C-1 carbon in starch adjacent to a substituted C-2 carbon.
[d] C2s means the substituted C-2 carbon in starch adjacent to a esterified C-3 carbon.
[e] Ca,t indicates the carbonyl carbon in LA near OH end group.
[f] Ca,i donates the main carbonyl carbon in LA.

The HMQC spectra of starch-g-PLA copolymer in Fig. 5 show the correlation peak of each carbon and its directly attached proton. The HMQC spectra enable us to assign the signals of starch-g-PLA copolymer more accurately.

The ^{13}C NMR spectrum of cornstarch as well as starch-g-PLA in Fig. 6 shows the glucopyranan carbons between 60 and 101 ppm. Contrast to extensive researches that have been carried out on the modification of starches, we found that some confusions still existed in the assignment of ^{13}C-shifts of starches and their derivatives, especially the signals corresponding to C-2, C-3 and C-5 carbons at the glycopyranan unit. Dicke et al. (2001) assigned the three peaks at 73.0 ppm, 70.5 ppm and 69.5 ppm respectively to C-5, C-3 and C-2 carbons with unsubstituted hydroxyl groups in the glucopyranan ring of starch. However, in contrast, Choi et al. (1999) attributed the signals at 73.4, 72.2 and 71.8 ppm respectively to the C-2, C-3 and C-5 carbons with unsubstituted hydroxyl groups at the glucopyranan unit in starch. ^{13}C-shifts of acetyl starch were also assigned ambiguously where the peak assignment of cellulose esters was used as reference (Heins et al., 1998). Contrast to the confused assignments, Perlin, Casu, and Koch (1970) and Mclntyre, Ho, and Vogel (1990) reported that the ^{13}C-chemical shifts were affected by the difference of the residue conformation of α-D-glucose and β-D-glucose. In their investigations, the signals corresponding to C-5, C-3 and C-2 carbons at β-D-glucose unit were set at about 76.5, 76.2 and 74.6 ppm, respectively, while the signals assigned to C-5, C-3 and C-2 carbons at α-D-glucose unit were centered at about 71.6, 73.3 and 71.9 ppm, respectively. Based on these reports and in coordinating with the analysis of our HMQC spectra and the published data of amylose, amylopectin and starch, we assigned the main ^{13}C-chemical shifts of the carbons

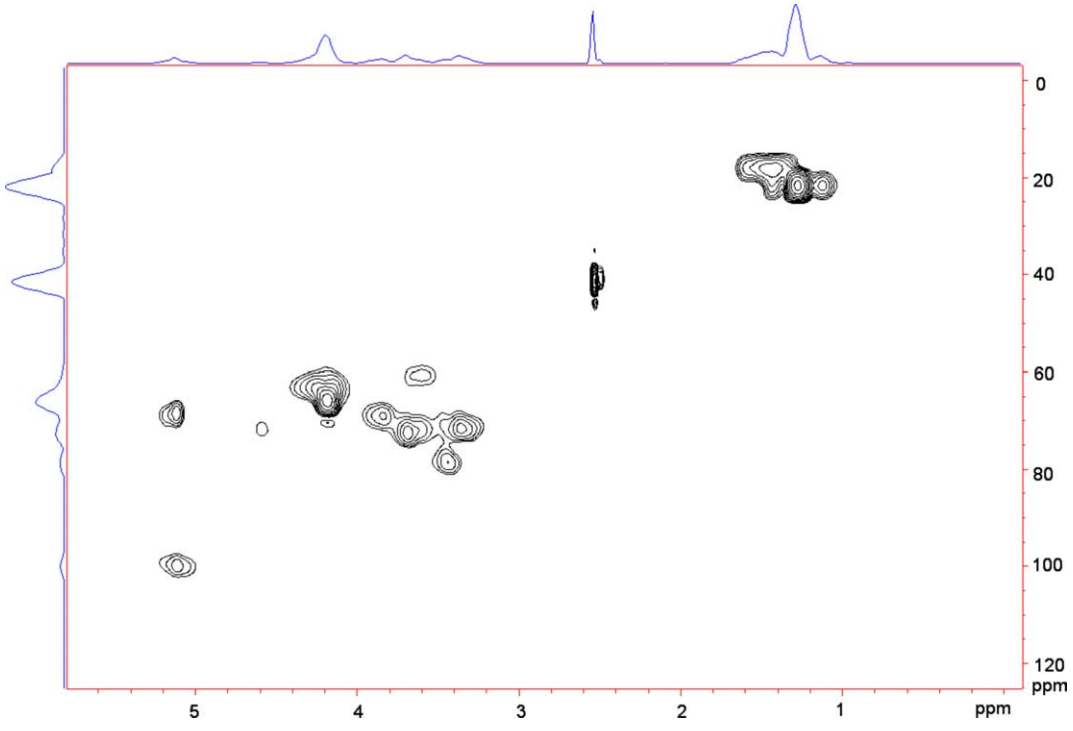

Fig. 5. Typical two-dimensional HMQC spectra of starch-g-PLA.

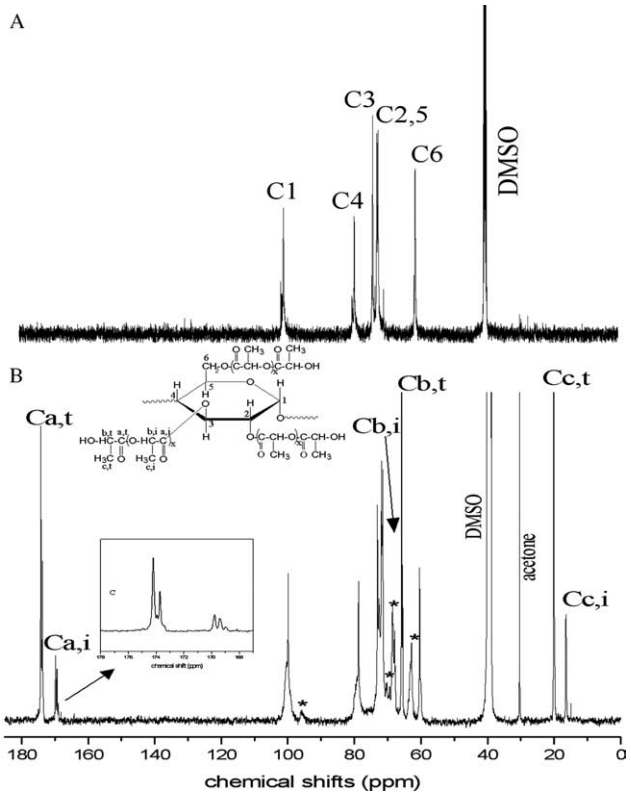

Fig. 6. ^{13}C NMR spectra of cornstarch (A), starch-g-PLA (B) and carbonyl carbon region of starch-g-PLA (C) in DMSO-d6. The latter two spectra are taken from quantitative ^{13}C NMR to obtain the microstructures of starch-g-PLA. The additional signals of ring carbon in starch-g-PLA are clarified by asterisk.

connecting to unsubstituted hydroxyl groups at 99.9 ppm to C-1, 78.8 ppm to C-4, 73.1 ppm to C-3, 71.9 ppm to C-2, 71.5 ppm to C-5, and 60.4 ppm to C-6 carbons, respectively. The relative minor signals separated from those of the main peaks at 100.3, 70.5, 72.6 and 60.6 ppm were attributed to C-1, C-4, C-5 and C-6 carbons at the nonreducing glucopyranan group at amylopectin moiety, respectively (Dais & Perlin, 1982; Peng & Perlin, 1987; Gagnaire et al., 1978; Nilsson et al., 1996; Arranz, Sanchez-Chaves, & Riofrio, 1987). In starch-g-PLA copolymer, it is found that the resonance intensities of the six ring carbons become bated comparing with that in the original starch (Fig. 6B). Besides the original resonance of the six starch ring carbons, additional signals at 95.7, 71.6, 70.5, 69.5, 68.6 and 62.8 ppm are presented and believed belonging to the carbons in a lactylated glucopyranan unit. It is known that the esterification of the hydroxyl group of glucopyranan compounds causes a resonance downfield shift of the carbon linked to an esterified hydroxyl group, while the resonance of the adjacent carbons shifted upfield. Therefore, the peak denoted by C-1′ at 95.7 ppm can be assigned to C-1 carbon adjacent to a substituted C-2 carbon. The signal designated as C-6s at 62.9 ppm may be attributed to C-6 carbon attached to an esterified hydroxyl group. Furthermore, the peak indicated as C5-s6 at 68.6 ppm can be assigned to C-5 carbon adjacent to an esterified C-6 carbon. These assignments are supported by the following facts: (1) the combined integral of the peaks of C-1 and C-1′ is almost equal to that of the peaks of C-6 and C-6s. (2) The integral of the peak of C5-s6 is identical to that of C-6s. The signal denoted by C4-s3 corresponding to C-4 adjacent to an esterified C-3 carbon can be set at 72.6 ppm. The minor signals at 71.6 and 70.6 ppm are respectively assigned to the esterified C-3 and C-2 carbons at glucoparanan unit in starch. More detailed information about the possible ^{13}C-signal assignments of starch-g-PLA is shown in Table 1.

Apart from the signals of starch ring carbons, we find the evidence of lactyl unit in three spectrum regions of 16.4–20.1, 65.6–67.9 and 169.4–174.2 ppm (Fig. 6B), arising from the methyl carbons, methene groups and carbonyl groups in LA moiety, respectively. Donabedian and McCarthy (1998) reported that the signals in the region of 173 and 20 ppm were indicative of successful acylation. However, according to Hiltunen et al. (1996) and our analysis of HMQC spectra, we conclude that the peaks at 173, 65.6 and 20 ppm should be attributed to the carbons in LA moiety near the end OH groups. The three separated peaks of carbonyl carbon in the region of 173 ppm as well as of 169 ppm (Fig. 6C) enable us to determine the position of lactyl unit at the glucopyranan ring. Since the integral of the peak C-6s should be identical to that of the terminal carbonyl carbon at C-6 position, and analogically, the integral of the peak C-1′ should be equal to that of the terminal carbonyl carbon at C-2 position, the signals corresponding to terminal carbonyl carbons at 174.2, 173.9 and 173.7 ppm can be assigned to the carbonyl carbons of lactyl unit at C-6, C-2 and C-3 position, respectively. The signals on the upfield shoulder of the peak at 173.7 ppm were attributed to the terminal carbonyl carbons at the nonreducing end of amylopectin. Like the terminal carbonyl absorptions, the heterogeneousness of the internal carbonyl absorptions is also resulted in by the substitution of lactate groups on starch backbone (Donabedian & McCarthy, 1998). Therefore, the peaks at 169.8, 169.4, and 169.0 ppm can be attributed to the internal carbonyl carbons in lactyl unit at C-6, C-2 and C-3 positions, respectively. These detailed assignments are also shown in Table 1.

3.3. Quantitative analysis of the microstructure of starch-g-PLA

Based on the peak assignments of starch ring carbons as well as the carbonyl carbons in LA moiety shown in Table 1, the detailed microstructure of starch-g-PLA is elucidated. The actual position of lactyl units at the glycopyranan ring, DS values and DP values are also analyzed by means of the quantitative ^{13}C NMR spectra.

DP, defined as the average length of PLA grafts on starch backbone, is calculated based on the area ratio of the terminal methyl carbon in LA moiety at 20.0 ppm and the internal methyl carbon in LA at 16.8 ppm to that of the terminal LA methyl carbon at 20.0 ppm, ascribed as follows:

$$\mathrm{DP} = \frac{[I(c,t) + I(c,i)]}{I(c,t)}$$

Where I(*c*,*t*) and I(*c*,*i*) are the NMR signal integrals of the terminal LA methyl carbon and the internal LA methyl carbon, respectively.

DS, defined as the average number of hydroxyl groups in starch substituted by lactyl units per glucopyranan residue of starch, can be obtained by

$$\mathrm{DS} = \frac{I(c,t)}{[I(st)/6]}$$

where I(*c*,*t*) is the NMR signal integral of the terminal LA methyl carbon, and I(*st*) is that of the six starch ring carbons. In this case, the relative mole ratio (MR) of LA and starch in the starch-g-PLA sample, defined as the average number of introduced lactyl units per glucopyranan residue of starch, is simply given by multiplying DP and DS:

$$\mathrm{MR} = \mathrm{DP} \times \mathrm{DS}$$

Detailed perspective of the graft structure of starch-g-PLA can be obtained with the assignments of the signals of glucopyranan units in starch-g-PLA. Based on the assignment, the relative DS at C-6 position denoted as DS6 can be obtained by

$$\mathrm{DS6} = \frac{I(6s)}{[I(6s) + I(6u)]}$$

where I(6s) and I(6u) represent the intensities of the C-6 carbon bearing an esterified hydroxyl group and C-6 carbon with an unsubstituted hydroxyl group, respectively.

Similarly, the relative DS at C-2 position designated as DS2 can be obtained by

$$\mathrm{DS2} = \frac{I(1')}{[I(1') + I(1u)]}$$

where I(1′) and I(1u) are the integrals of the C-1 carbon adjacent to a C-2 carbon with a substituted hydroxyl group and the C-1 carbon adjacent to the C-2 carbon with an unsubstituted hydroxyl group, respectively.

It is a little difficult to determine directly the relative DS at C-3 position (DS3) by the above method because of the peak overlap. However, since the hydroxyl groups at C-4 carbon in the nonreducing end of amylopectin are especially minor, and the esterification of hydroxyl groups at the glucopyranan ring of starch is occurred mainly at C-2, C-3 and C-6 position, DS3 can be approximately obtained by

$$\mathrm{DS3} = \mathrm{DS} - \mathrm{DS2} - \mathrm{DS6}$$

The results of the structural parameters of starch-g-PLA calculated from the starch ring carbons are displayed in Table 2.

Besides the calculation from starch ring carbons, the heterogeneous carbonyl absorptions of LA moiety, on the other side, can also provide detailed information on the graft efficiency of PLA grafts at C-2, C-3 and C-6 carbons. Based on this deduction, the DS values for the carbons at the glycopyranan unit can be calculated by

$$\mathrm{DS2} = \frac{I(2a,t)}{[I(st)/6]} \qquad \mathrm{DS3} = \frac{I(3a,t)}{[I(st)/6]} \qquad \mathrm{DS6} = \frac{I(6a,t)}{[I(st)/6]}$$

Table 2
Structure parameters of starch-g-PLA calculated from quantitative ^{13}C NMR spectra of starch-g-PLA

	Structure parameters[a]	Structure parameters[b]
DP	1.33	1.32
DS	0.86	0.87
MR(LA/starch)	1.14/1	1.15/1
DS6	0.56	0.52
DS3	0.22	0.26
DS2	0.08	0.09
DS4		0.05
MF6 (DS6/DS)	0.65/1	0.60/1
MF3 (DS3/DS)	0.26/1	0.30/1
MF2 (DS2/DS)	0.09/1	0.10/1
DP6		1.25
DP3		1.14
DP2		2.27
DPcal		1.32

[a] Calculation based on the ^{13}C NMR spectroscopy of starch ring carbons.
[b] Calculation according to the spectra of carbonyl carbons in LA moiety.

where I(2a,t), I(3a,t) and I(6a,t) represent the integrals of the terminal carbonyl group at C-2, C-3 and C-6 position, respectively. In the above calculation DS4 is included in DS3. In correspondence, however, the effect of DS4 on DS3 can be estimated by calculating DS4 from the terminal carbonyl absorption at C-4 position as follows:

$$\mathrm{DS4} = \frac{I(4a,t)}{[I(st)/6]}$$

where I(4a,t) represents the integral of the terminal carbonyl group at C-4 position. For our system, the degree of substitution at C-4 position in the nonreducing end of amylopectin is 0.05. Accordingly, the effect on DS3 is minor.

The mole fraction of the substitution at C-2, C-3 and C-6 carbons (MF2, MF3 and MF6) in starch-g-PLA copolymer can be obtained by simple calculation using these DS values (Table 2).

Furthermore, the assignments of the heterogeneous internal and terminal carbonyl carbons can also be used for the evaluation of DP at the site of C-2, C-3 and C-6 carbons. In the calculation of DP at C-3 position from the carbonyl carbons in LA moiety, DP at C-4 position in the nonreducing end of amylopectin is also included. The corresponding values can be obtained by

$$\mathrm{DP2} = \frac{[I(2a,t) + I(2a,i)]}{I(2a,t)} \qquad \mathrm{DP3} = \frac{[I(3a,t) + I(3a,i)]}{I(3a,t)}$$

$$\mathrm{DP6} = \frac{[I(6a,t) + I(6a,i)]}{I(6a,t)}$$

where I(2a,i), I(3a,i) and I(6a,i) denote the integrals of the internal carbonyl groups at C-2, C-3 and C-6 carbons, respectively.

The total DP of PLA grafts can be obtained by algebraic addition of DP6, DP2 and DP3. All the results on the structure parameters of the starch-g-PLA sample calculated from the carbonyl absorptions of LA moiety are summarized in Table 2.

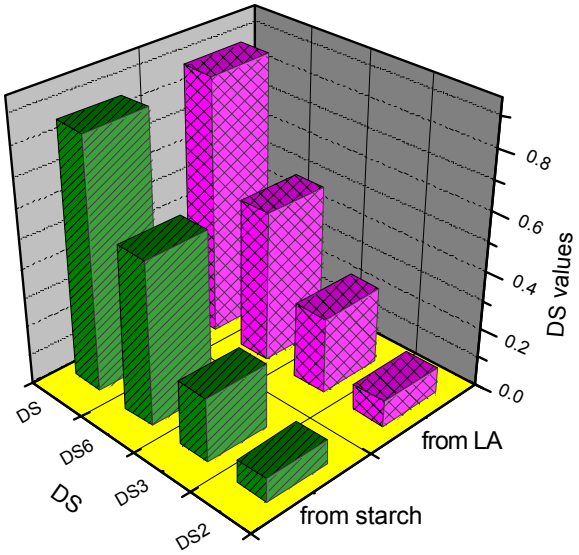

Fig. 7. DS values at individual starch ring carbons calculated from the region of starch ring carbons as well as from the carbonyl carbons in LA moiety.

It is worth to note that the DS values calculated from the starch ring carbons are identical to that from the carbonyl carbons in LA moiety (see Fig. 7). The result is also an indication of the correct assignment of the NMR resonance peaks in starch-g-PLA copolymer.

In order to compare the relative reactivity of the three-hydroxyl groups in starch, we should notice that at the branch point of amylopectin there is a C6–O–C1 ether linkage instead of a hydroxyl group. In our system, the degree of the amylopectin branch is 0.16 calculated from ^{1}H-NMR spectrum of cornstarch. Therefore, the initiation reactivity of hydroxyl group at C-6 carbon can be calculated as follows:

$$IR6 = DS6/0.84$$

Fig. 8 shows the relative reactivity of three hydroxyl groups in glucopyranan unit of cornstarch. It is seen that the relative reactivity of three hydroxyl groups decreases in the order of C-6 > C-3 > C-2. The reactivity result is in consistent with that in

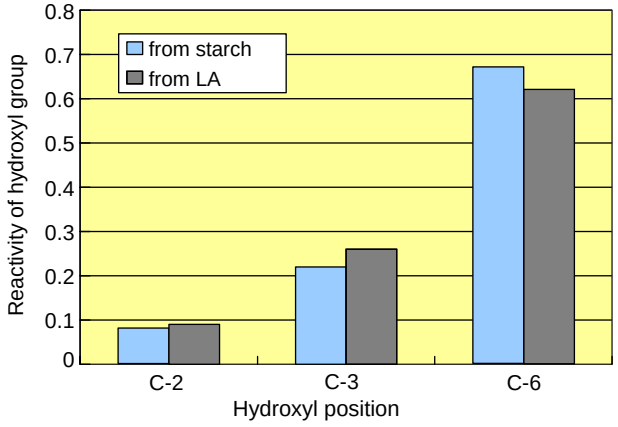

Fig. 8. The reactivity of hydroxyl groups at individual starch ring positions calculated from the region of starch ring carbons as well as from the carbonyl carbons in LA moiety.

the previous reports on the esterification of amylose (Arranz et al., 1987; Arranz & Sanchez-Chaves, 1995). According to the ROP mechanism proposed for starch grafted with lactic acid in this paper, it is believed that the steric hindrance might be the main reason for inducing the differences in the reactivity of three hydroxyl groups in glucopyranan unit of cornstarch. The primary hydroxyl group at C-6 position has the smallest steric effect comparing with that of the secondary hydroxyl groups at C-2 and C-3 carbons. While the difference in the reactivity between the two secondary hydroxyl groups at C-2 and C-3 positions might be due to the higher steric hindrance of the hydroxyl group at C-2 carbon than that at C-3 carbon because of the more energetically favourable conformation (Arranz et al., 1987).

Korhonen et al. (2001) found that the difference in the reactivity of hydroxyl groups of co-initiators resulted in PLA with a biomodal molecular weight distribution in the case of $Sn(Oct)_2$ as initiator. They supposed that random breakages of the polymer chains were induced by trace lactic acid or other impurities. The intermolecular transesterification or random breakages resulted in the decrease of the molecular weight. The average length of PLA grafts on individual cornstarch carbon of starch-g-PLA was shown in Fig. 9. The average length of PLA grafts is 1.33 indicating that PLA grafts with less than two units are grafted to starch. PLA grafts with only one unit might be the result of the occurrence of breakages or intermolecular transesterification in PLA grafts. Moreover, the DP values increase in the order of C-3 < C-6 < C-2. It seems that the phenomenon shows a close relation with the reactivity of the hydroxyl groups. The average length of PLA grafts at C-6 position is 1.25, implying that the breakages or intermolecular transesterification take place in about 75% of PLA grafts at C-6 position. Similarly, the average length of PLA spacer at C-3 position is 1.14, indicating that nearly 86% of PLA grafts at C-3 position contains only one lactate unit. Both values of the average length of PLA grafts at C-6 and C-3 carbons are less than 2 contrasting to that at C-2 position which is 2.27. The reason resulting in the shorter PLA grafts at C-3 position with respect to that at C-6 position cannot be fairly understood at the present stage. The approximate value of DP3 where DP4 is included might be one of the reasons for inducing the shorter PLA grafts at C-3 position.

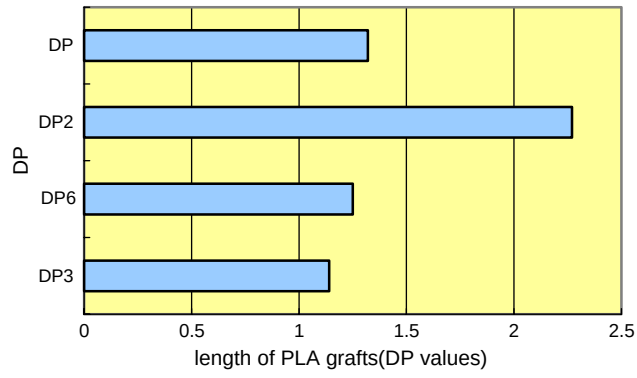

Fig. 9. The average length of PLA grafts at individual starch ring carbons.

4. Conclusion

Starch grafted copolymer with PLA was formed by a direct reaction of starch and lactic acid in aqueous media in the presence of $Sn(Oct)_2$. It is confirmed that the conjugation of PLA to the backbone of starch is via the ROP of lactide formed in situ in the reaction system. Analysis of ^{13}C NMR spectra indicates that the ROP of lactide takes place through acyl-oxygen cleavage co-initiated by hydroxyl group at glucopyranan unit, yielding PLA grafts with a hydroxyl terminus. The assignments of the resonance in ^{1}H- and ^{13}C NMR spectra of the copolymer are clarified. The positions of lactyl units at the glucopyranan ring as well as the corresponding distribution are determined. The DS values of the graft copolymer calculated based on ^{13}C NMR spectra of starch ring carbons are identical to that of carbonyl carbons in LA moiety. The reactivity of hydroxyl groups at glucopyranan ring decreases in the order of C-6 > C-3 > C-2. While the average length of PLA grafts increases in the order of C-3 < C-6 < C-2. The phenomena are proposed due to the difference of the steric hindrance of hydroxyl groups at glucopyranan unit in starch.

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