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Chemical modification of pullulan by isocyanate compounds

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Abstract

The modified pullulan derivatives having *N*-phenylurethane and *N*-hexylurethane groups in the side-chain (PI-PL and HI-PL) have been synthesized by the reactions of pullulan with phenyl isocyanate (PIC) and hexyl isocyanate (HIC), respectively. The structure of the derivatives was characterized by gel permeation chromatography, IR and ^{1}H NMR spectroscopy. From the measurement of differential scanning calorimetry of PI-PL and HI-PL with various degrees of addition (DA) in the range of 0–3, it was revealed that both the derivatives showed a clear glass transition temperature (T_g), which decreased with the increasing DA of PIC and HIC. The pullulan derivatives were water-resistant and had good solubility in general organic solvents. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Pullulan; Isocyanate; Chemical modification

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1. Introduction

Pullulan is a biodegradable water-soluble polysaccharide extracellularly produced by growing a fungus-like yeast, *Aureobasidium pullulans* or *Pullularia pullulans*, commonly called "black yeast" on starch syrups in about 70% yield [1,2]. The structure of pullulan is proposed to consist predominantly of maltotriose units, i.e. units of three α-1,4-linked glucose molecules, which are polymerized in a linear fashion via α-1,6-linkages, as shown in Fig. 1. Pullulan is a promising material on the environmental aspects because it is biodegradable and produced from plant-based starch or sugars regenerated by natural photosynthesis of carbon dioxide and water. However, pullulan itself is difficult to process by conventional melt processing techniques without the use of processing aids, as is obvious from the result that pullulan begins to degrade thermally at approximately 250°C without a clear appearance of glass transition temperature (T_g) and melting temperature (T_m) on heating. Also, pullulan has poor water-resistance and toughness (elongation of the 30 μm film, ca. 3.0%). Although many studies on the chemical modification of pullulan such as oxidation [3], carbonation [4], succinoylation [5], palmitoyl and cholesteyl derivatives [6–8] and cyanoethylation [9] etc. for uses as prodrug carrier [3–5], host for protein guests [6–8], and organic electroluminescent devices [9] etc. have been reported, little effort has been devoted to its use as plastics. Donabedian et al. reported that the acetylated pullulan prepared by the reaction of pullulan with acetic anhydride and pyridine shows T_g around 160°C [10], and that pullulan 6-hydroxyhexanoates and pullulan 6-dilactates prepared by the reactions with ϵ-caprolactone and L-lactide show T_m and T_g [11], respectively. The present paper describes the synthesis of the modified pullulan derivatives with urethane groups in the side chain by the reaction of pullulan with isocyanate compounds and their properties. Our attention is focused on the thermoplasticity of the modified derivatives.

2. Experimental

2.1. Materials

Pullulan, PF-20 grade supplied by Hayashibara Biochemical Laboratories, Inc. ($\bar{M}_n = 124,000$ g/mol, $\bar{M}_w = 331,000$ g/mol, measured by GPC method using water as an eluent and pullulan standards) was dried under vacuum at 70°C for 24 h before use. The reagent grade dimethyl sulfoxide (DMSO) was dried over molecular sieves of 5 Å for three days. The reagent grade phenyl isocyanate (PIC) and hexyl isocyanate (HIC) were freshly vacuum-distilled before use. Other reagents, acetone, toluene, and ethanol (Kanto Chemicals Co. Ltd, Japan) were used without further purification.

2.2. Synthesis of phenyl isocyanate-modified pullulan derivative (PI-PL)

Pullulan 5.00 g (hydroxyl group 92.5 mmol), DMSO

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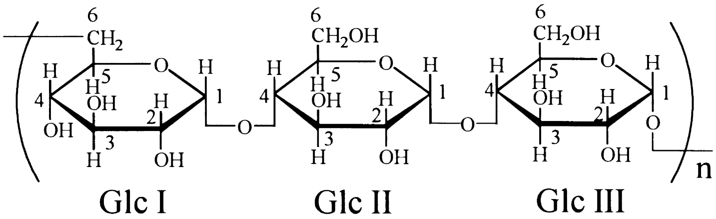


Fig. 1. Structure of pullulan.

150 ml, and toluene 50 ml were added to a three necked round bottom flask equipped with a Dean stark trap, reflux condenser and mechanical stirrer under nitrogen. After the solution was azeotropically dehydrated under vacuum (25 Torr, 75–80°C) for 5 h, PIC 14.7 g (0.123 mol) was added dropwise to the solution at room temperature within 0.5 h. After the mixture was reacted for 24 h at 65°C, the reaction mixture was poured into 1 l water. The precipitate was filtered and washed with ethanol several times. The obtained wet cake was dried at 70°C in vacuo to give PI-PL (observed degree of addition (DA) 3.0 by NMR method) 13.8 g in 85.9% yield.

The variation of the amount of feed PIC, the DA and the yield of the products are summarized in Table 1.

2.3. Synthesis of hexyl isocyanate-modified pullulan derivative (HI-PL)

Pullulan 5.00 g (hydroxyl group 92.5 mmol), DMSO 150 ml, and toluene 50 ml were added to a three necked round bottom flask equipped with a Dean stark trap, reflux condenser and mechanical stirrer under nitrogen. After the solution was azeotropically dehydrated under vacuum (25 Torr, 75–80°C) for 5 h, hexyl isocyanate 15.3 g (0.123 mol) was added dropwise to the solution at room temperature within 0.5 h. After the mixture was reacted for 24 h at 65°C, the reaction mixture was poured into 1 l water. The precipitate was filtered and washed with a mixed solvent of acetone/water (volume ratio: 1:1) several times. The obtained wet cake was dried at 70°C in vacuo to give HI-PL (obs. DA 2.9 by gel permeation chromatography (GPC) method) 11.8 g in 73.1% yield.

The variation of the amount of feed HIC, the DA and the yield of the products are summarized in Table 2.

2.4. Characterization

The IR spectra of the modified pullulans were measured on a FT-IR 8100 spectrometer (Shimazu Co. Ltd, Japan) by KBr method. GPC analysis was performed using a Shimazu LC-9A instrument equipped with two linear PLgel 5 μm MIXED-D columns (Polymer Laboratories Ltd), using tetrahydrofuran as the eluent at a pressure of 32 kgf/cm^2, the elution rate was 0.5 ml/min and the detector was the refractive index type. Polystyrene standards with a low polydispersity were used to generate a calibration curve for the molecular weight determination. ^{1}H NMR spectra were recorded on a Brucker AMX-400 spectrometer at 400 MHz, using DMSO-d_6 and tetramethysilane (TMS) as a solvent and an internal standard. The degree of addition (DA) of the modified pullulans was determined by GPC and/or NMR methods. The GPC measurements of crude products obtained by the concentration of the reaction mixtures at 70°C in vacuo showed that the products contain 1,3-diphenylurea and 1,3-dihexylurea which probably formed by the reactions of PIC and HIC with water, respectively, in addition to their oligomers as by-products. The quantitative analysis of each component of the by-product was performed in comparison with the peak area of standard 1,3-diphenylurea or 1,3-dihexylurea in the GPC chart, assuming that the oligomers have the same sensitivity to the refractive index type detector as the urea derivatives. The DA by GPC method was calculated, assuming that all the isocyanate which was not converted to the by-product

Table 1
Synthetic data of PI-PL (because the samples of theo. DA 0.5 and 1.0 were not completely soluble in THF, the measurement of molecular weight could not be done)

Feed PIC[a]	theo. DA	obs. DA		Yield (%)	M_w	M_n	M_w/M_n
		(NMR)	(GPC)				
0.167	0.5	0.2	0.5	78	–	–	–
0.333	1.0	0.9	0.9	85	–	–	–
0.500	1.5	1.3	1.2	93	383,000	220,000	1.74
0.667	2.0	2.0	1.7	77	437,000	220,000	1.98
0.833	2.5	2.2	1.9	70	422,000	221,000	1.91
1.333	3.0	3.0	2.9	86	333,000	206,000	1.61

[a] Molar ratio of PIC to hydroxyl group of pullulan.

Table 2
Synthetic data of HI-PL (because the samples of theo. DA 0.5–2.0 were not completely soluble in THF, the measurement of molecular weight could not be done. Also, because the samples of theo. DA 2.3–3.0 were not completely soluble to DMSO-d_6, the measurements of DA by NMR method could not be done)

Feed HIC[a]	theo. DA	obs. DA		Yield (%)	M_w	M_n	M_w/M_n
		(NMR)	(GPC)				
0.166	0.5	0.4	0.5	87	–	–	–
0.333	1.0	1.0	0.9	92	–	–	–
0.667	2.0	1.4	1.1	–	–	–	–
0.767	2.3	–	1.8	88	232,000	103,000	2.25
1.000	3.0	–	2.5	75	234,000	97,000	2.40
1.333	3.0	–	2.9	73	226,000	77,000	2.92

[a] Molar ratio of PIC to hydroxyl group of pullulan.

reacted with pullulan. Regarding NMR method, DA was calculated from the comparison between the integration values of the protons of pullulan moiety and those of phenyl or hexyl substituents. For PI-PL, using the integration value of C–H of the benzene ring (A) observed at 6.5–7.5 ppm and that of C–H and O–H of the pullulan moiety (B) observed at 3–6 ppm, the DA is expressed by $DA = 30A/(3A + 15B)$. For HI-PL, using the integration value of C_5H_{11}–CH_2– of the hexyl group (C) observed at 0.6–1.5 ppm and that of C–H and O–H of the pullulan moiety and C_5H_7–CH_2– of hexyl group (D) observed at 2.6–5.6 ppm, the DA is expressed by $DA = 30C/(33D - 3C)$.

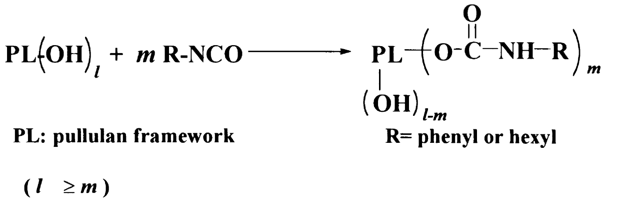
Differential scanning calorimetry (DSC) measurements were performed using a Perkin–Elmer Pyris 1 DSC instrument with a heating rate of 20°C/min in a nitrogen atmosphere. Thermogravimetric analysis (TGA) was performed on a Perkin–Elmer TGA-7 instrument, using a heating rate of 20°C/min in a nitrogen atmosphere.

Tensile properties of the films prepared by the casting of DMSO solutions were examined using an Autograph AGS-500C (Shimazu Co. Ltd, Japan) based on the standard method for testing tensile properties of films and sheets (JIS K7127). Span length was 50 mm, thickness of films was ca. 100 μm, and testing speed was 10 mm/min.

3. Results and discussion

3.1. Synthesis and characterization of isocyanate-modified pullulans

Isocyanate-modified pullulans were synthesized by the reaction of pullulan with PIC or HIC in DMSO/toluene (Fig. 2). This reaction is very sensitive to the presence of water, because of the high reactivity of isocyanate and water. Therefore, the water contained in the solution was azeotropically removed with toluene before the reaction. In spite of dehydration, the crude product obtained by the concentration of the reaction mixture contained considerable amount of 1,3-diphenylurea or 1,3-dihexylurea in addition to its oligomer, as is obvious from the GPC chart (Fig. 3). The urea derivative may be formed by the reaction of PIC or HIC with residual water accompanying decarboxylation. Also the oligomer may be formed by the subsequent reaction of the urea derivative with PIC or HIC, and the repeating reactions. Therefore, for purification, the reaction mixtures of pullulan with PIC and HIC were poured into water and the obtained precipitates were washed several times with ethanol and acetone/water, respectively. The synthetic data of the isocyanate-modified pullulans are summarized in Tables 1 and 2. The IR spectra of the modified pullulans showed new absorption peaks at ca. 1720 and 1550 cm^{-1} related to stretching vibration of the carbonyl group of the urethane bond and the bending vibration of N–H, respectively. (Fig. 4). Regarding ^1H NMR spectrum, all the proton signals of pullulan in DMSO-d_6 were already assigned in detail by Brunel et al. [12]. Pullulan is a linear glucan consisting of a repeating trisaccharide units, containing two $\alpha(1 \rightarrow 4)$ linkages and one $\alpha(1 \rightarrow 6)$ linkage. The glucoside units in the repeating trisaccharide are labelled Glc I, Glc II and Glc III, respectively, as shown in Fig. 1. For the PI-PL with obs. DA 0.2 (NMR method), the decrease of OH-6 of Glc III at 4.4 ppm is obviously appeared, indicating that the reactivity of the hydroxyl group of pullulan to PIC may be the order of OH-6 of Glc III > OH-6 of Glc II q OH-2,3,4 (Fig. 5). Fig. 6 shows the ^1H NMR spectra of pullulan, PI-PL with obs. DA 0.9 (NMR) and HI-PL with obs. DA 1.0 (NMR). For the PI-PL, the proton signals of N–H of the urethane group and those of the phenyl group appeared at ca. 9.6 and 6.5–7.5 ppm, respectively. For the HI-PL, the proton signals of C_5H_{11}–CH_2– of the hexyl group and those of N–H of the urethane group are observed at 0.6–1.5 and ca. 7.0 ppm appeared, respectively. These results indicate that the urethane bond was certainly formed by the reaction of



$$PL(OH)_l + m\,R\text{-}NCO \longrightarrow PL\underset{(OH)_{l-m}}{-}\left(O\text{-}\overset{O}{\overset{\|}{C}}\text{-}NH\text{-}R\right)_m$$

Fig. 2. Synthetic scheme of PI-PL and HI-PL.

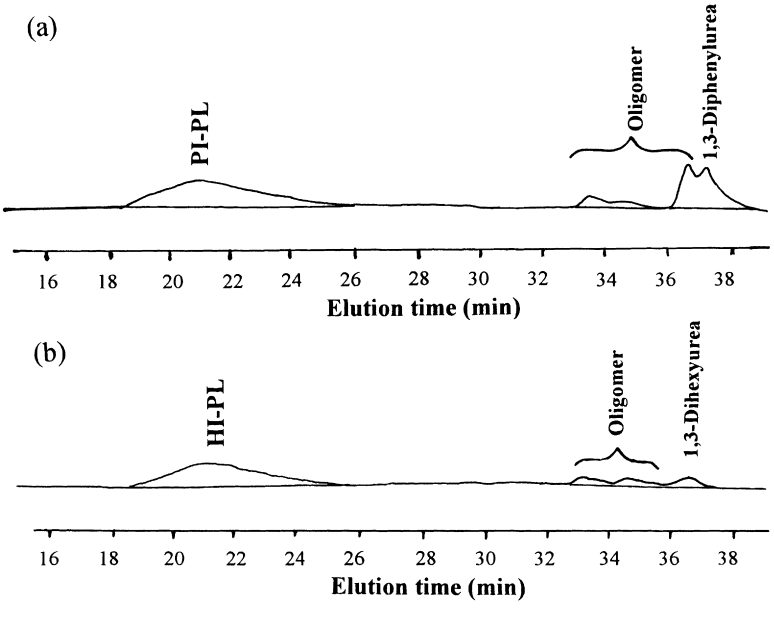


Fig. 3. GPC charts of the crude products obtained by the reaction of pullulan with (a) PIC and (b) HIC.

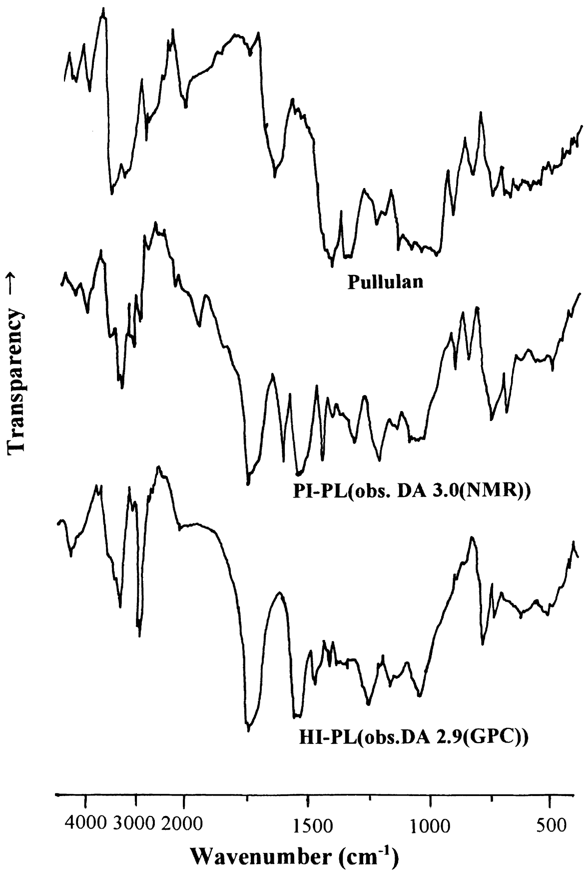


Fig. 4. IR spectra of pullulan, PI-PL with obs. DA 3.0 (NMR) and HI-PL with obs. DA2.9 (GPC).

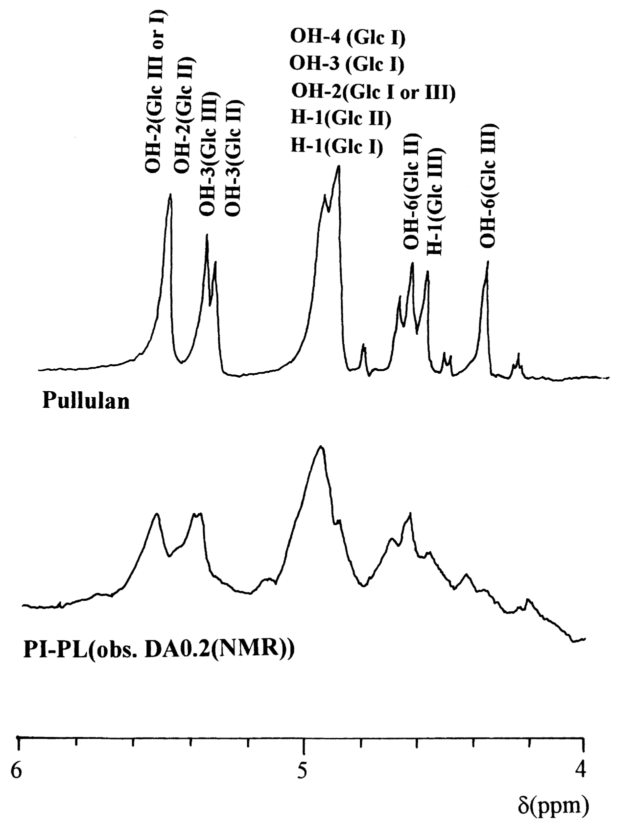


Fig. 5. ^{1}H NMR spectra of pullulan and PI-PL with obs. DA 0.2 (NMR) in the OH proton region.

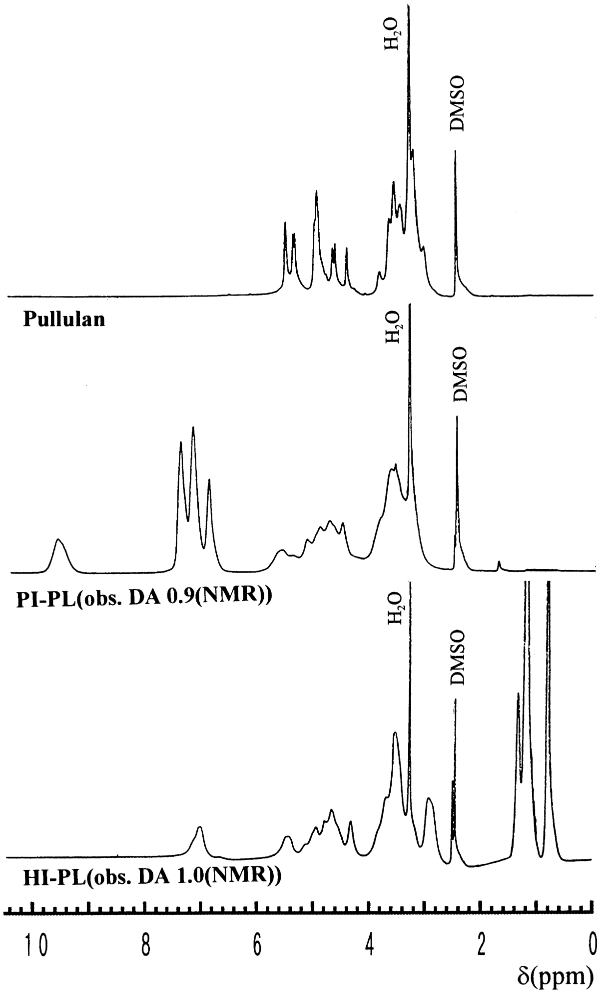


Fig. 6. ^{1}H NMR spectra of pullulan, PI-PL with obs. DA 0.9 (NMR) and HI-PL with obs. DA 1.0 (NMR).

isocyanate and the hydroxyl group of pullulan for PI-PL and HI-PL. The DA values of the products were determined by the NMR method and the GPC method. As shown in Tables 1 and 2, the samples with various DA values in the range of 0–3 were successfully obtained and there was no great difference between the two methods. The M_w of PI-PL (333,000–437,000) were higher than that of pullulan (331,000), indicating that low molecular weight products with a higher DA which are more soluble in ethanol were removed on washing with ethanol. On the other hand, the M_w of HI-PL (226,000–234,000) were lower than that of pullulan, indicating that higher molecular weight products with a lower DA which are more soluble in acetone/water were removed on washing with acetone/water. These assumptions should support the idea that the change of molecular weight is pronounced in the samples of lower yield.

Table 3
Solubility of PI-PL in various solvents (solubility was determined after immersing the sample 5 mg in each solvent 0.5 ml for 24 h; W: Soluble, K: Swollen, ×: Insoluble)

Solvent	obs. DA						
	0.0	0.2	0.9	1.3	2.0	2.2	3.0
Ethyl alcohol	×	×	×	K	K	K	K
Acetone	×	×	×	W	W	W	W
Ethyl acetate	×	×	×	K	W	W	W
Diethyl ether	×	×	×	×	K	K	W
Chloroform	×	×	×	×	K	K	W
Tetrahydrofuran	×	×	K	W	W	W	W
DMSO	W	W	W	W	W	W	W
N,*N*-dimethylformamide	W	W	W	W	W	W	W
Toluene	×	×	×	×	×	×	×
Pyridine	W	W	W	W	W	W	W
Water	W	W	K	×	×	×	×

Table 4
Solubility of HI-PL in various solvents (solubility was determined after immersing the sample 5 mg in each solvent 0.5 ml for 24 h; W: Soluble, K: Swollen, ×: Insoluble)

Solvents	obs. DA						
	0.0	0.5	1.0	1.4	1.8	2.5	2.9
Ethyl alcohol	×	K	W	K	K	K	K
Acetone	×	×	K	K	W	W	W
Ethyl acetate	×	×	×	×	W	W	W
Diethyl ether	×	×	×	×	W	W	W
Chloroform	×	×	×	×	W	W	W
Tetrahydrofuran	×	K	W	W	W	W	W
DMSO	W	W	W	W	K	K	×
N,*N*-dimethylformamide	W	W	W	W	W	W	W
Toluene	×	×	×	×	K	W	W
Pyridine	W	W	W	W	W	W	W
Water	W	×	×	×	×	×	×

3.2. Solubility, and thermal and mechanical properties of isocyanate-modified pullulans

Solubility of PI-PL and HI-PL with various DA values is summarized in Tables 3 and 4. PI-PL and HI-PL with the DS value not less than 0.9 and 0.5 were insoluble in water, respectively. Regarding the solubility toward the general organic solvents, for example, the PI-PL with DS not less than 1.3 was soluble in acetone, and the HI-PL with DS not less than 1.8 was soluble in ethyl acetate, diethyl ether and chloroform.

Table 5
Thermal and mechanical properties of PI-PL

obs. DA(NMR)	T_g (°C)[a]	T_d (°C)[b]	Tensile strength (MPa)[c]
0.0	–	295	44
0.2	217	282	
0.9	204	277	
1.3	201	268	23
2.0	201	299	
2.2	198	297	
3.0	196	291	12

[a] Measured by DSC at a heating rate of 20°C/min under nitrogen.

[b] Measured by TG at a heating rate of 20°C/min under nitrogen. Decomposition temperature (T_d) was defined as a 5% weight loss temperature.

[c] All films were dried for 12 h under vacuum at 70°C before measurement.

Table 6
Thermal properties of HI-PL

obs. DA	T_g (°C)[a]	T_d (°C)[b]	Tensile strength (MPa)[c]
0.0 (NMR)	–	295	44
0.4 (NMR)	198	307	
1.0 (NMR)	167	302	32
1.4 (NMR)	157	296	
1.8 (GPC)	136	283	14
2.5 (GPC)	122	279	
2.9 (GPC)	119	274	10

[a] Measured by DSC at a heating rate of 20°C/min under nitrogen.

[b] Measured by TG at a heating rate of 20°C/min under nitrogen. Decomposition temperature (T_d) was defined as a 5% weight loss temperature.

[c] All films were dried for 12 h under vacuum at 70°C before measurement.

The glass transition temperature (T_g) and 5% weight loss temperature (T_d) of the modified pullulans are summarized in Tables 5 and 6. The native pullulan showed no clear T_g in our DSC experimental condition, although it is reported that pullulan has a T_g at 178°C [13]. It is considered that the endothermal behaviour above ca. 230°C for pullulan should be related to the start of the decomposition. On the other hand, all the modified pullulans exhibited a clear T_g. The T_g decreased with increasing DA values for both PI-PL and HI-PL (Figs. 7 and 8). HI-PL had a lower T_g than PI-PL, as compared with the samples with a similar DA value. As a whole, regarding T_d, HIC modification causes a slight decrease of T_d, while PIC modification almost does not affect the heat resistance (Tables 5 and 6). The HI-PL with obs. DA 2.9 (GPC) has a T_g of 119°C and T_d of 274°C, indicating that it has a sufficient processing window in melt processing.

Tensile properties of the PI-PL and HI-PL films prepared by the casting of DMSO solution are also summarized in Tables 5 and 6, respectively. The tensile strength decreased with increasing DA for all the modified pullulans. The tensile strength of the native pullulan film allowed to stand for three days became 4 MPa, because of the moisture absorption. On the other hand, the tensile strength of all the modified pullulans did not change after standing for several days. Elongation at break of the modified pullulans has an almost similar value to that of the native pullulan (ca. 3%). The biodegradability of the modified pullulan derivatives is now under investigation.

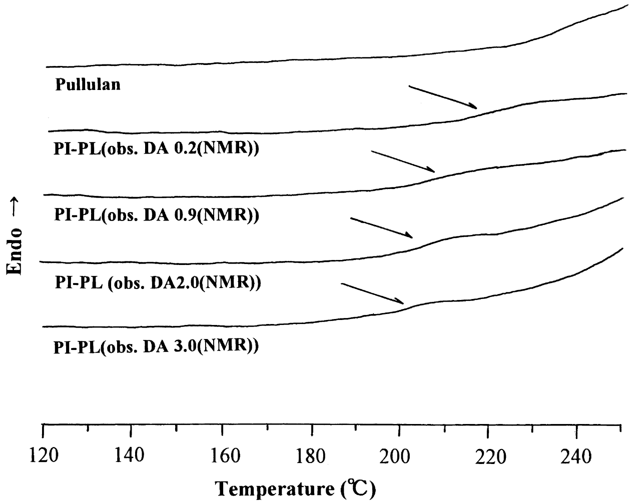


Fig. 7. DSC charts of PI-PL with various DA.

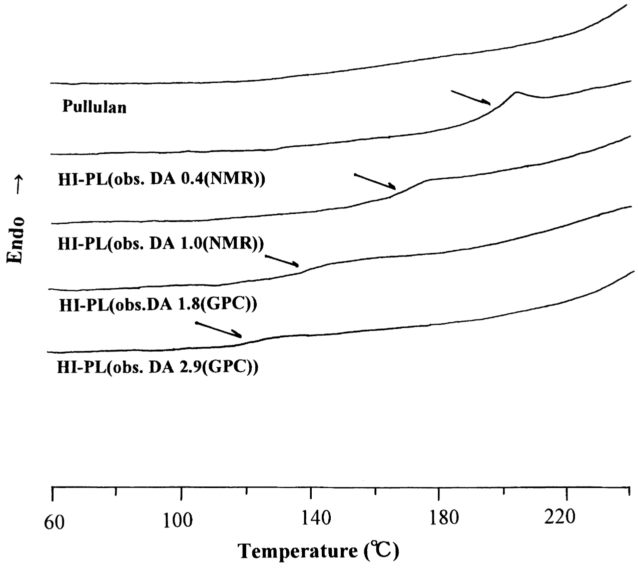


Fig. 8. DSC charts of HI-PL with various DA.

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