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PREPARATION AND ENZYMATIC HYDROLYSIS OF BLOCK COPOLYMER CONSISTING OF OLIGOCHITIN AND POLY(PROPYLENE GLYCOL)

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ABSTRACT

Block copolymer **2** consisting of alternating oligochitin units and poly(propylene glycol) (PPG) units was prepared by the dehexanoylation of a protected parent block copolymer **1**. Complete dehexanoylation was achieved with sodium methoxide in a mixed solvent of methanol and DMSO at room temperature to give block copolymer **2**. The structure of **2** was determined by ^{1}H-NMR and IR spectroscopies. The thermal behavior of **2** was determined by TG analysis. The enzymatic hydrolysis of **2** by chitinase was examined in acetate buffer solution. The hydrolysis was followed by using high performance liquid chromatographic (HPLC) analysis. The majority of the chitin units in **2** was hydrolyzed to yield *N*-acetyl-D-glucosamine and *N,N'*-diacetylchitobiose. No reaction occurred without chitinase.

INTRODUCTION

Block copolymers consisting of poly- or oligosaccharides are of considerable interest because of such unique properties as elasticity, water sorption, and biocompatibility [1–3]. It has been previously demonstrated that enzymatic degradable block copolymers can be prepared by the incorporation of oligomeric cellulose or

amylose blocks into the main chain of the copolymer [4–6]. We recently reported that oligodihexanoylchitin having hydroxy groups at both ends was prepared by the acid hydrolysis of a parent dihexanoylchitin in a mixed solvent of acetic acid and concentrated hydrochloric acid [7]. The product oligomer was copolymerized with poly(propylene glycol) (PPG) using 4,4′-methylene(diphenyl isocyanate) (MDI) as a coupling reagent to give the block copolymer **1** [7]. The oligomeric protected chitin block was introduced into the main chain of the copolymer by this copolymerization.

In this paper we report the modification of **1** to block copolymer **2** consisting of alternating oligochitin units and PPG units by a complete dehexanoylation (Scheme 1). The thermal behavior and enzymatic biodegradability of **2** were also examined.

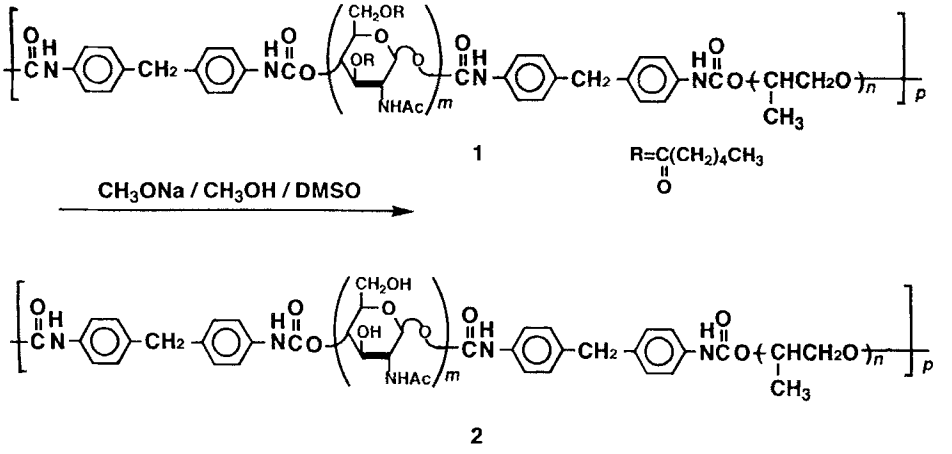
EXPERIMENTAL

Materials

The protected block copolymer **1** was prepared by the copolymerization of oligodihexanoylchitin (M_n = 7200) with PPG (M_n = 3000) as previously reported [7]. The number-average molecular weight of **1** was 52,000 as determined by gel permeation chromatographic (GPC) analysis. Sodium methoxide was a commercial reagent used without further purification. Chitinase from *Bacillus* sp (activity: 0.045 units/mg) was purchased from Wako Pure Chemical Ind. (Tokyo) and employed without further purification. The solvents dimethylsulfoxide (DMSO) and methanol were purified by distillation over CaH_2 and Mg metal, respectively.

Dehexanoylation of 1

To a solution of **1** (0.16 g) in DMSO (3.0 mL) was added to sodium methoxide (0.05 g) in methanol solution (2.0 mL) under argon at 0°C, and the mixture was stirred for 6 hours at room temperature. The reaction mixture was neutralized



SCHEME 1.

by acetic acid, and 10 mL water was added to precipitate the product. The product was isolated by filtration and dried in vacuo to give 0.07 g of **2** (65% yield).

Enzymatic Hydrolysis of 2

To a suspension of **2** (0.0134 g) in acetate buffer (pH 6.0, 1.5 mL) was added chitinase (0.0030 g), and the mixture was stirred at 40°C for 41 hours. The water-insoluble part in the reaction mixture was separated by filtration to give 0.0085 g of **5** and analyzed by ^{1}H-NMR spectroscopy. The filtrate was directly analyzed by HPLC, which showed the exclusive formation of *N*-acetyl-D-glucosamine (**3**) and *N,N'*-diacetylchitobiose (**4**). Additionally, the filtrate was concentrated and poured into a large amount of acetone to precipitate the products. The precipitate products were isolated by decantation and dried in vacuo to give 0.0043 g of the powdery materials. The materials were confirmed as a mixture of **3** and **4** (3.6:1) by ^{1}H-NMR analysis.

Measurements

^{1}H-NMR spectra were recorded on a Jeol EX-270 spectrometer. IR spectra were recorded on a Horiba FT-200 spectrometer. HPLC analyses were performed by using a Hitachi 655A-11 apparatus with UV detector under the following conditions: Merck LiChrospher RP-18(e) (5 μm, 4.0 mm × 250 mm) column with water eluent at a flow rate of 1.0 mL/min. Thermal analyses were performed on a Seiko SSC 5200 TG-DTA 220 thermal analyzer at a heating rate of 10°C/min.

RESULTS AND DISCUSSION

Preparation of Block Copolymer 2

As previously reported, the block copolymerization of oligodihexanoylchitin with PPG successfully took place to give block copolymer **1** [7]. Dehexanoylation of **1** was carried out in a mixed solvent of DMSO and methanol in the presence of sodium methoxide at room temperature under argon. After 6 hours the product was isolated as a white powdery material. ^{1}H-NMR and IR analyses of the product confirmed that complete dehexanoylation took place to give block copolymer **2**. The ^{1}H-NMR spectra of the product (DMSO-d_6) is shown in Fig. 1(B). Small Peaks b at δ 6.9–7.4 are due to aromatic protons. Broad peaks at δ 3.0–5.0 are ascribable to sugar protons. Peak c, due to methylene protons between aromatics, overlaps with the sugar peaks; the chemical shift of Peak c is uncertain. Broad Peaks d at around δ 3.4 are ascribable to methylene and methine protons of PPG. Singlet Peaks f and i at δ 1.80 and 0.95 are assignable to methyl protons of acetamide and PPG, respectively. The disappearance of the peaks due to hexanoyl protons of **1** in Fig. 1(A) strongly supports that complete dehexanoylation was achieved. The IR spectrum of the product in Fig. 2(B) exhibits absorptions at 1720 cm^{-1} ($\nu_{C=O}$ of urethane) and 1650 cm^{-1} ($\nu_{C=O}$ of amide). The absorption at 1745 cm^{-1} due to the ester carbonyl of **1** in Fig. 2(A) has completely disappeared, suggesting complete dehexanoylation. These spectroscopic data indicate that the structure of the product

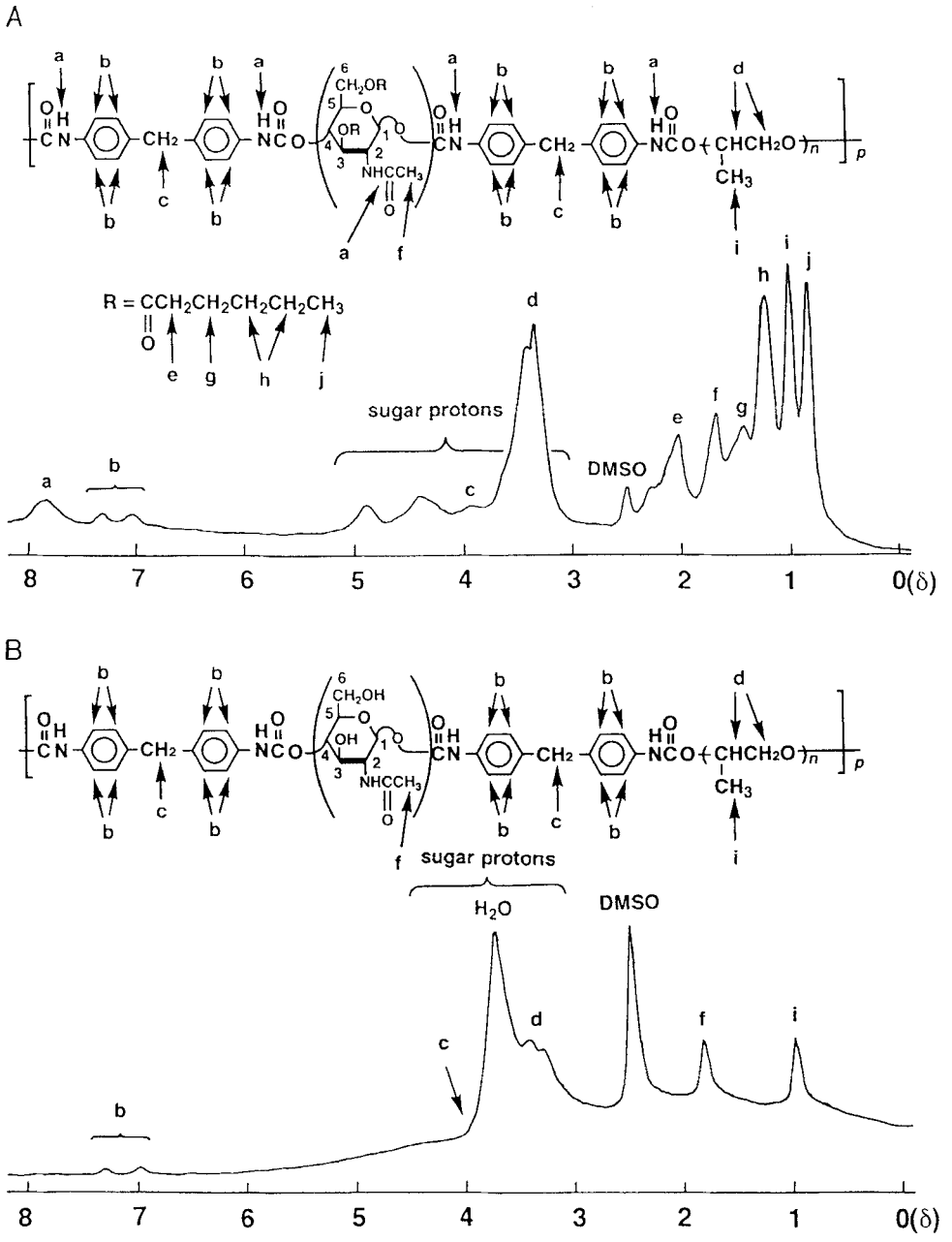


FIG. 1. ^{1}H-NMR spectra of block copolymer **1** (A) and block copolymer **2** (B) in DMSO-d_6.

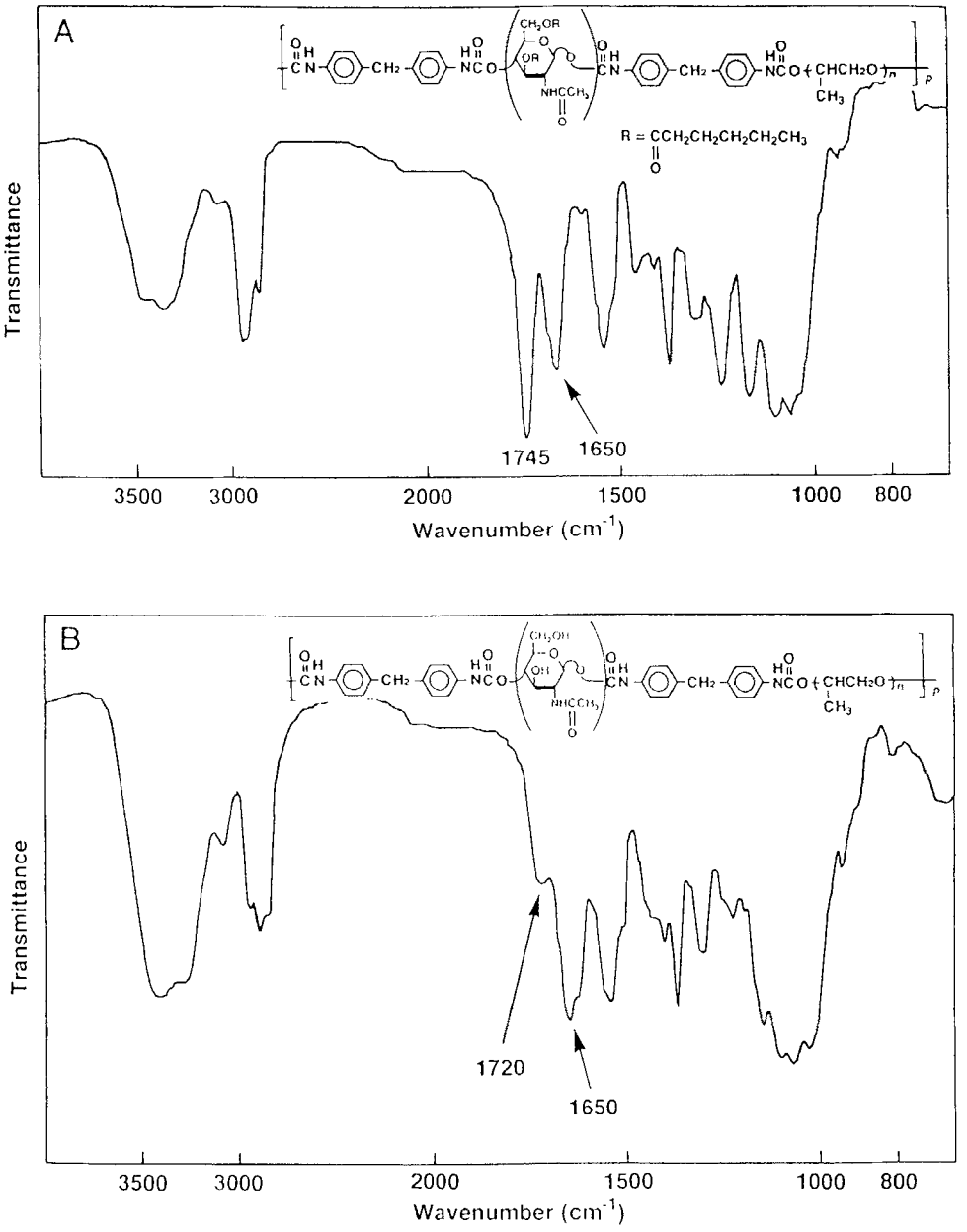


FIG. 2. IR spectra of block copolymer **1** (A) and block copolymer **2** (B).

is block copolymer **2**. Block copolymer **2** is soluble in DMSO but insoluble in other common organic solvents and water.

Thermal Behavior of 2

The thermal behavior of **2** was examined by thermogravimetry (TG). A typical trace for block copolymer **2** in nitrogen is shown in Fig. 3. Block copolymer **2** showed an onset of weight loss at 224°C. At 428°C, **2** had lost approximately 70% of its weight. Chitin and PPG showed significant weight losses at 275–428 and 210–394°C, respectively. These data indicate that the degradation of both the oligochitin units and the PPG units in **2** occurred between 224 and 428°C. In addition, **2** has a higher thermal stability than **1**, which showed a 70% weight loss at 190–377°C.

Enzymatic Hydrolysis of 2

In order to confirm the hydrolysis of the oligochitin unit in **2**, enzymatic digestion by chitinase from *Bacillus* sp in acetate buffer (pH 6.0) was carried out. After stirring for 41 hours at 40°C, the water-insoluble part in the reaction mixture was separated by filtration, and the filtrate was analyzed as a mixture of *N*-acetyl-D-glucosamine (**3**) and *N,N'*-diacetylchitobiose (**4**) by HPLC analysis (Scheme 2). In order to confirm the products further, the filtrate was concentrated and poured into a large amount of acetone to precipitate the products. The ^{1}H-NMR spectrum of the products in D_2O supported the formation of **3** and **4** in a 3.6:1 ratio. Furthermore, the ^{1}H-NMR spectrum was identical with that of a mixture of the authentic samples of **3** and **4**. On the other hand, the water-insoluble part separated from the reaction mixture was soluble in DMSO and *N,N*-dimethylformamide (DMF) but

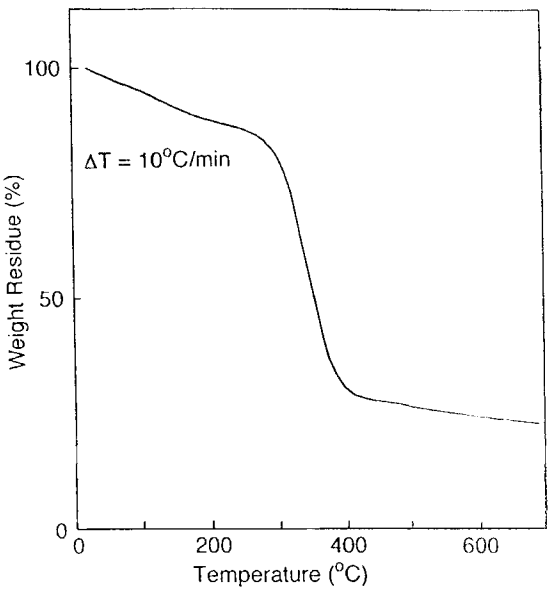
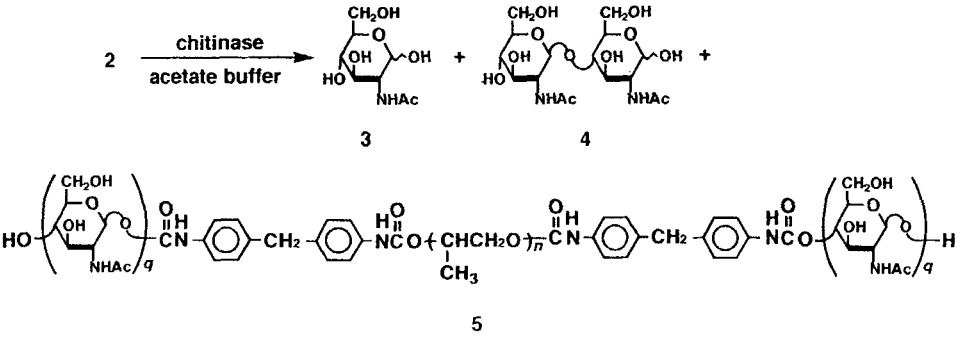


FIG. 3. TG trace of block copolymer **2** in nitrogen.



SCHEME 2.

insoluble in other common organic solvents. The ^{1}H-NMR spectrum (DMSO-d_6) showed mainly peaks due to the PPG structure accompanied by very small peaks assigned to aromatic and sugar protons. From the above data, the structure of **5** for the water-insoluble part can be assumed. The average of the q value was 3.3 as calculated by the integral ratio between peaks due to aromatic and acetamide groups. This value suggests that the majority of the oligochitin unit in **2** was hydrolyzed by enzyme, because the DP value of the oligochitin unit in **2** was around 18. Without chitinase, **2** was not hydrolyzed at all under the same reaction conditions.

CONCLUSION

We synthesized block copolymer **2** consisting of alternating oligochitin units and PPG units by the complete deprotection of block copolymer **1**. This is the first example of a block copolymer containing chitin units in the main chain. Additionally, the enzymatic hydrolysis of **2** by chitinase was confirmed.

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