

Effect of hydrophilic and hydrophobic monomers grafting on microbial poly(3-hydroxybutyrate)

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ABSTRACT

The radiation-induced graft polymerization of hydrophilic [sodium *p*-styrene sulfonate (SSS), acrylic acid (AAc)] and hydrophobic [styrene (St), methyl acrylate (MAAc)] monomers onto microbial poly(3-hydroxybutyrate) (PHB) powder was performed and the characteristics of the both grafting films obtained after remolding were compared. The grafting of various monomers onto the PHB powder increased with reaction time. The degree of grafting (X_g) onto the PHB by these various monomers followed the order St, SSS, MAAc and AAc. The PHB films onto which were grafted St, SSS, MAAc and AAc were characterized by measuring the contact angle, the adsorption of enzyme PHB depolymerase, and the biodegradability, as well as by differential scanning calorimetry. Both hydrophobic and hydrophilic monomers affected the contact angle of grafted PHB film with St, SSS, MAAc and AAc monomers. However, the adsorption of enzyme on the grafted PHB film increased with the amount of hydrophilic monomer and degree of grafting. Consequently, the enzyme PHB depolymerase easily approached the PHB film when the hydrophilic groups were grafted onto its surface. The enzymatic degradation of grafted PHB films with hydrophilic monomer proceeded more quickly than that of hydrophobic monomers.

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1. Introduction

The microbial polyesters poly(3-hydroxybutyrate) P(3HB), is a biocompatible and biodegradable thermoplastic polymer that is produced by various bacteria in nature as an intracellular carbon and energy source (Anderson and Dawes, 1990; Doi, 1990). The degradable P(3HB) had attracted considerable attention for its many agricultural, industrial, and medical applications (Doi, 1990). However, the brittleness and stiffness of PHB limit its range of practical applications, and the enzymatic degradability of PHB must be controlled to enable utilization.

Three techniques have been applied to overcome this shortcoming of PHB. One of the approaches is to biosynthesize copolymers that contain poly(hydroxyalkanoate) (PHA) units. The second is to prepare the blend polymers of PHB and other chemical

synthesized polymers. The third is radiation grafting onto PHB. Another application of radiation graft polymerization is developmental research into the separation and refinement of proteins to separate functional materials (Miyoshi *et al.*, 2005). For environmental reasons, materials are being developed as graft adsorbents to remove toxic metals from industrial waste waters and the harmful constituents of the atmosphere (Seko *et al.*, 2003, 2004; Shiraishi *et al.*, 2003). Research into the recovery of useful rare metals from seawater and hot springs is currently being undertaken (Seko *et al.*, 2005). The graft adsorbent has been used to recover rare metals such as scandium (Sc), vanadium (V) and arsenic (As) from the hot springs of Kusatsu in the Gunma Prefecture. These graft adsorbents are improved by changing the functional groups on the graft chains to promote the recovery of required metals.

This authors' research group recently succeeded in controlling the enzymatic degradability of PHB using the radiation graft copolymerization method (Bahari *et al.*, 1997, 1998; Cakmakli *et al.*, 2001; Mitomo *et al.*, 1995, 1996). In an earlier work our group described grafted hydrophilic monomers (2-hydroxyethyl methacrylate (HEMA) (Mitomo *et al.*, 1995), acrylic acid (AAc) (Mitomo *et al.*, 1996) and the hydrophobic monomers, styrene (St) (Bahari *et al.*, 1997, 1998; Cakmakli *et al.*, 2001) and methyl methacrylate (MMA)) onto PHB. These experiments indicated that while grafting

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Abbreviations: P(3HB), poly(3-hydroxybutyrate); SSS, sodium *p*-styrene sulfonate; AAc, acrylic acid; St, styrene; MAAc, methyl acrylate; X_g , degree of grafting; Sc, scandium; V, vanadium; As, arsenic; HEMA, 2-hydroxyethyl methacrylate; MMA, methyl methacrylate; BOD, biochemical oxygen demand; KGy, irradiation dose; Torr, unit of pressure; ΔH_m , enthalpy of melting; $(\Delta H_m)_{corr}$, corrected enthalpy of melting; T_m , melting point.

PHB into the hydrophobic MMA sharply reduced its enzymatic degradability, introducing hydrophilic HEMA increased enzymatic degradability (Mitomo et al., 1995). Accordingly, the effect of the hydrophilicity of the grafting chains on the enzymatic degradation of the PHB was approximately determined.

In this work, onto microbial PHB was grafted various hydrophilic monomers, including sodium *p*-styrene sulfonate (SSS) and acrylic acid (AAc), and hydrophobic monomers, such as styrene (St) and methyl acrylate (MAAc), using γ -rays. The thermal properties of grafted PHB were determined. Furthermore, the enzymatic degradability, contact angle and enzymatic adsorption of the grafted samples were discussed.

2. Materials and methods

2.1. Preparation of materials

PHB was purchased from Aldrich Chemical Co., Ltd. (USA). PHB was purified as follows: PHB (20 g) was dissolved in chloroform (1000 mL) and then poured into a solvent that consisted of *n*-hexane and methanol (2000 mL, 1:1 vol%). The precipitated PHB powder was then isolated by filtration and dried under vacuum. The mean particle size of the obtained PHB powder was about 20 μ m. The St monomer, purchased from Kanto Chemical Co., Inc., was used after the inhibitor was removed at graft proceeding. The monomers of SSS, AAc (purchased from Tokyo Kasei Kogyo Co., Ltd.) and MAAc (purchased from Kanto Chemical Co., Inc.) were used without further purification.

2.2. Radiation-induced graft polymerization on PHB powder

The PHB-g-SSS, PHB-g-St, PHB-g-AAc, and PHB-g-MAAc powders were prepared by the pre-irradiation before radiation-induced graft polymerization. The PHB powder (2.0 g) was placed on one side of an H-shaped glass ampoule that was sealed at reduced pressure (10^{-3} Torr). The PHB was then pre-irradiated with ^{60}Co γ -rays (dose rate = 10 kGy/h) at 10 kGy in the glass ampoule at -70°C . Following irradiation, the monomer (10 mL) was poured into the opposite side of the ampoule, and it was resealed and evacuated (10^{-3} Torr). The degree of grafting (X_g) given by the following equation (Basuki et al., 2003):

$$X_g(\%) = (W_g - W_0) \times \frac{100}{W_0} \quad (1)$$

where W_0 and W_g are the weights of the PHB powder before and after the graft polymerization, respectively.

The grafting reactions of the St, SSS, AAc and MAAc monomers proceeded in a temperature-controlled water bath at 60°C for 10 min, 30 min and 3 h before the grafted powders were immersed in a solvent to terminate the grafting reaction and to remove the homopolymers. The concentration of monomer in the St was denoted 100 wt.% solution in this study. The monomer concentration of the SSS solution was adjusted to 0.5 M using water as the solvent and the monomer concentrations of the AAc and MAAc solutions were adjusted to 30 and 10 wt.%, respectively, using methanol as the solvent.

2.3. Analysis

The thermal properties of the PHB powder and film were investigated using a Shimadzu DSC-50 (Japan). About 2 mg of the sample was packed in an aluminum pan and heated from 25°C to 190°C at a rate of $10^\circ\text{C}/\text{min}$ in a 30 mL/min nitrogen flow. The enthalpy of melting (ΔH_m) was determined by DSC from the area

under the endothermic peak. The temperature was calibrated using highly pure benzoic acid standards.

The ΔH_m value was corrected using the weight fraction of PHB in the grafted samples and the following equation (Mitomo et al., 1995, 1996) (corrected enthalpy of melting (ΔH_m)_{corr.}).

$$\frac{\Delta H_m}{(\Delta H_m)_{\text{corr.}}} = \frac{100 - X_g}{100} \quad (2)$$

The contact angles of various monomers on the grafted PHB films were measured using a contact-angle meter (Kyowa Scientific Co., Ltd.). The contact angles were determined using a drop of 0.05 M NaOH solution at room temperature.

The grafted PHB powder was formed into a film by hot-pressing at 190°C . The obtained films were 150 μ m thick and were isothermally crystallized at 90°C for 1 week before use. The enzymatic degradation of the irradiated PHB films was evaluated at 37°C in 0.1 M phosphate buffer (pH 7.4). PHB depolymerase purified from *Ralstonia pickettii* T1 (Yamada et al., 1993) was used. The irradiated PHB films (150- μ m thick) were cut into squares of 10 mm \times 10 mm and then placed in small test tubes that contained 1.0 mL of buffer solution. The reactions were initiated by adding 4 μ g of PHB depolymerase. The weight loss of the film was periodically measured after it was removed and washed using methanol and distilled water. In this work, the weight loss ($\text{mg}/\text{cm}^2 \text{ h}$) was calculated from the weight after 48 h of enzymatic degradation.

The enzymatic adsorption test involved a U-3310UV spectrophotometer from HITACHI. The grafted PHB film adsorbed enzyme at 10°C in 0.1 M phosphate buffer (pH 7.4). The grafted PHB films (initial weight, about 10 mg; film dimensions, 10 mm \times 10 mm; film thickness, 0.09 mm) were placed in small test tubes that contained 1.0 mL of the buffer. The reactions were initiated by adding aqueous PHB depolymerase (1 μ g). The reaction solutions were incubated with shaking. The film of enzyme-adsorbing PHB was then periodically removed, and the reduced solution (1 μ L) was moved to another buffer solution that contained the PHB matrix at 37°C . The reaction solution was assayed by measuring the drop in turbidity at 650 nm and 37°C .

3. Results and discussion

3.1. Grafting PHB powder with acrylic acid

Acrylic acid (AAc) was grafted onto the PHB powder with 20 wt.% (\blacktriangle), 30 wt.% (\circ), and 50 wt.% (\bullet) monomer solutions at 10 kGy. Fig. 1 plots the relationship between the reaction time and

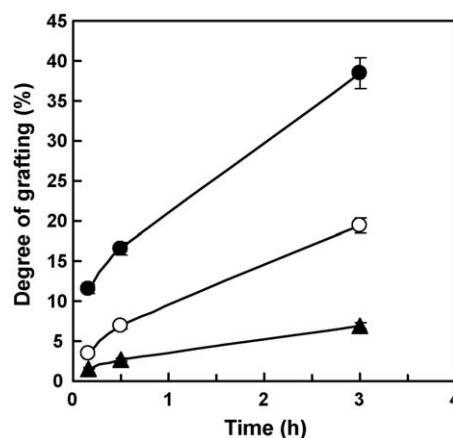


Fig. 1. Relationship between reaction time and degree of grafting (X_g). Monomer concentration: (\blacktriangle) 20 wt.% AAc solution, (\circ) 30 wt.% AAc solution, and (\bullet): 50 wt.% AAc solution.

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