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

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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 (Anderson and Dawes, 1990; Doi, 1990). The brittleness and stiffness of P(3HB) limit its range of practical applications, and the enzymatic degradability of P(3HB) must be controlled to enable utilization.

Three techniques have been applied to overcome this shortcoming of P(3HB). 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 polymerization method (Bahari et al., 1997, 1998; Cakmakli et al., 2001; Mitomo et al., 1995, 1996). In an earlier work our group described graft 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...
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 degradability 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 μm. 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 60Co 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 (Xg) given by the following equation (Basuki et al., 2003):

\[ X_g(\%) = \frac{W_g - W_o}{W_o} \times \frac{100}{W_g} \]  

where \( W_o \) 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 °C/min in a 30 mL/min nitrogen flow. The enthalpy of melting (\( \Delta H_m \)) was determined by DSC from the area under the endothermic peak. The temperature was calibrated using highly pure benzoic acid standards.

The \( \Delta H_m \) 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 (\( \Delta H_m^{corr} \)).

\[ \frac{\Delta H_m^{corr}}{\Delta H_m} = \frac{100 - X_g}{100} \]  

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 μm 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 fromRalstonia picketii T1 (Yamada et al., 1993) was used. The irradiated PHB films (150-μm thick) were cut into squares of 10 mm × 10 mm and then placed in small test tubes that contained 1.0 mL of buffer solution. The reactions were initiated by adding 4 μg 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 (mg/cm² 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 × 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 μg). The reaction solutions were incubated with shaking. The film of enzyme–adsorbing PHB was then periodically removed, and the reduced solution (1 μL) 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.% (▲), 30 wt.% (○), and 50 wt.% (●) 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 (Xg). Monomer concentration: (▲) 20 wt.% AAc solution, (○) 30 wt.% AAc solution, and (●): 50 wt.% AAc solution.](image-url)
degree of grafting. The $X_g$ of PHB increased with the reaction time on the grafted PHB powder for each concentrations of monomer solution. Moreover, the monomer concentration affected the degree of grafting. The $X_g$ of PHB increased with the monomer concentration (●: 50 wt.% AAc > ○: 30 wt.% AAc > ▲: 20 wt.% AAc) over a reaction time of 3 h.

3.2. Hydrophobic/hydrophilic monomers grafted on PHB powder

To evaluate the effect of the grafting of hydrophobic/hydrophilic monomers onto the PHB powder, St (100 wt.%; ●), SSS (0.5 M; ○), AAc (30 wt.%; ○), and MAAc (10 wt.%; ●) were grafted onto the PHB powder using various concentrations of the monomer solutions. Fig. 2 plots the relationship between the reaction time and the degree of grafting. The grafting ratios of grafted PHB powder to various monomers increased with the reaction time to 3 h. The degree of grafting onto the PHB by these grafted PHB powders to various monomers decreased in the order St, SSS, MAAc, and AAc.

Grafting ratio and thermal properties of various PHB grafted monomer (St, SSS, and MAAc) films were degraded using the enzyme PHB depolymerase. Fig. 3 plots the relationship between $X_g$ (PHB-g-St (●) and PHB-g-SSS (○)) and weight loss after 48 h of enzymatic degradation. The weight loss of PHB-g-St significantly declined and that of PHB-g-SSS substantially increased as $X_g$ increased. This difference between the weight loss characteristics of the two grafted films was related to the presence or absence of the hydrophilic side chains on the graft chains, indicating that the grafting of St (PHB-g-St) and SSS (PHB-g-SSS) onto PHB powders affected their enzymatic degradability, although St and SSS have similar chemical structures, they have different hydrophilic properties.

Table 1 presents the grafting rates and thermal properties of the various PHB and PHB-grafted monomers (St, SSS, AAc, and MAAc) films. The melting point ($T_m$) and corrected enthalpies of melting ($\Delta H_m^{corr}$) of all grafted PHB samples were almost independent of the grafting ratio, suggesting that the graft chains barely affected the crystalline structure.

3.3. Enzymatic degradation of PHB film with various grafted monomers

The hot-pressed PHB and various grafted monomer (St, SSS, MAAc, and AAc) films were degraded using the enzyme PHB depolymerase. Fig. 3 plots the relationship between $X_g$ (PHB-g-St (●) and PHB-g-SSS (○)) and weight loss after 48 h of enzymatic degradation. The weight loss of PHB-g-St significantly declined and that of PHB-g-SSS substantially increased as $X_g$ increased. This difference between the weight loss characteristics of the two grafted films was related to the presence or absence of the hydrophilic side chains on the graft chains, indicating that the grafting of St (PHB-g-St) and SSS (PHB-g-SSS) onto PHB powders affected their enzymatic degradability, although St and SSS have similar chemical structures, they have different hydrophilic properties.

Fig. 4 plots the relationship between $X_g$ (PHB-g-AAc (○) and PHB-g-MAAc (●)) and weight loss after 48 h of enzymatic degradation. The difference between the enzymatic degradability of the PHB powder to which was grafted AAc (PHB-g-AAc) and that to which was grafted MAAc (PHB-g-MAAc) was examined. These results demonstrate that the weight loss of PHB-g-MAAc was lower...
than that of the PHB film, while that of PHB-g-AAc was higher, in each case to an extent that increased with the \( X_p \) of the samples: the degradation of grafted PHB-g-AAc polymer increased with the amount of hydrophilic monomer (AAc) after grafting, while that of grafted PHB-g-MAAc polymer declined as the amount of hydrophobic monomer (MAAc) increased after grafting. Similar results were obtained, as indicated above, for the grafting of St and SSS monomers onto PHB powder.

### 3.4. Contact angle of PHB film grafted various monomers

**Fig. 5** plots the contact angles between the PHB films to which had been grafted various monomers and the monomers themselves. The contact angles of PHB-g-AAc (\( \bigcirc \)) and PHB-g-SSS (\( \bigcirc \)) films were markedly reduced by increasing \( X_p \) perhaps because the surface of the PHB film was completely covered with the grafted acrylic acid or styrenesulfonate. However, the contact angles of the PHB-g-MAAc (\( \bullet \)) and PHB-g-St (\( \square \)) films significantly increased with \( X_p \). As described above, the grafting of hydrophilic monomer increased the enzymatic degradability of grafted PHB. Therefore, the grafting chains were homogenously distributed in the remolded film, suggesting that the hydrophobic property on the surface of the grafted PHB film influenced the contact angle of the grafting PHB film. Our research group has been detecting the cross-section of remolded PHB-g-AAc film using the SEM-EDX technique to observe the distribution of adsorbed elements (Wada et al., 2006). The surface of the remolded graft PHB sample was almost covered with the grafted monomer. The region close to the surface of the PHB film was overcrowded with graft chains as \( X_p \) was increased (data not shown).

### 3.5. Adsorption of enzyme on PHB-g-AAc film

**Fig. 6** plots the adsorption of the enzyme on the PHB-g-AAc film with various degrees of grafting. The adsorption increased with grafting, suggesting that an enzyme easily approaches the film when a hydrophilic group is added to a graft chain, indicating that the grafting of a hydrophilic chain to the PHB film increased its degradability.

### 4. Conclusions

The grafting of various monomers to the PHB powder increased with the monomer concentration and reaction time. The degree of grafting onto the PHB by these various monomers decreased in the order St, SSS, MAAc, and AAc.

The thermal properties of the PHB-g-St, PHB-g-SSS, PHB-g-MAAc and PHB-g-AAc films were very similar. A comparison of the enzymatic degradability of the pair of PHB-g-St and PHB-g-SSS films with the pair of PHB-g-MAAc and PHB-g-AAc films demonstrated that the presence of hydrophilic groups in the grafting chains affected the enzymatic degradability of the grafted PHB films.

The grafted PHB film was hydrophilic because of the grafting of hydrophilic monomers. Therefore, the enzyme PHB depolymerase easily approached the surface of the PHB film. Accordingly, the enzymatic degradability of these grafted PHB films exceeded that of films to which were grafted with hydrophobic monomers.

The contact angles of PHB grafted with various monomer films were influenced by the hydrophilic or hydrophobic character of the monomers. The adsorption of the enzyme on the PHB-g-AAc film increased with the degree of grafting, indicating that the enzyme PHB depolymerase easily approached the surface of the PHB film and that the hydrophilic chain grafted onto the PHB film improved its degradability.

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### References


