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Properties and enzymatic degradation of poly(acrylic acid) grafted polyhydroxyalkanoate films by plasma-initiated polymerization

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ABSTRACT

Using plasma-initiated polymerization, poly(acrylic acid) (PAA) was grafted onto the film surfaces of polyhydroxyalkanoate (PHA), poly[(*R*)-3-hydroxybutyrate] (P(3HB)), and two kinds of poly[(*R*)-3-hydroxybutyrate-*co*-4-hydroxybutyrate] (P(3HB-*co*-4HB)) with 4.7 mol% or 9.4 mol% of 4HB content. The graft ratio of PAA on the film surface increased drastically with acrylic acid concentration over 8%. Attenuated total reflection Fourier transform infrared spectroscopy demonstrated that the PAA grafted on the film surface was at least 70 nm thick. Mechanical and surface properties of PHA films grafted by PAA (PAA–PHA) were analyzed by tensile test, contact angle test, and scanning electron microscopy. While mechanical properties before and after plasma treatment remained unchanged, surface properties changed from hydrophobic to hydrophilic. Enzymatic degradation of PAA–PHA films was performed using an extracellular PHB depolymerase purified from *Ralstonia pickettii* T1. The rate of enzymatic degradation was inhibited by PAA graft. However, the rate of enzymatic degradability was accelerated by exposing ungrafted polymer to the enzyme solution, when a PAA-grafted film was divided into several pieces.

1. Introduction

Polyhydroxyalkanoate (PHA), including poly[(*R*)-3-hydroxy butyrate] (P(3HB)) and its copolymers, is a family of chiral aliphatic polyesters synthesized from recyclable carbon sources such as sugar and vegetable oil and is biodegradable in a natural environment [1]. The biodegradability, crystal structure, mechanical and thermal properties of the films and fibers of P(3HB) [2–4] and its copolymers, such as poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyvalerate] (P(3HB-*co*-3HV)) [5,6], poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyhexanoate] (P(3HB-*co*-3HH)) [7,8], and poly[(*R*)-3-hydroxybutyrate-*co*-4-hydroxybutyrate] P(3HB-*co*-4HB) [9–12], have been synthesized using recombinant *Escherichia coli* and studied in depth.

Some extracellular polyhydroxybutyrate (PHB) depolymerases were isolated from soil, rivers, and seawaters, and their primary and three-dimensional structures were analyzed [13–15]. The characterizations of the structure genes revealed that PHB depolymerases were organized with catalytic, linker, and substrate-biding domains. The enzymatic degradation starts first at the binding of PHB depolymerase on the material surface, and then hydrolysis occurs at the amorphous region and subsequently at the crystal region. In our previous studies, it was revealed that the rate of enzymatic degradation was affected by the content of the second monomer unit, crystallinity, lamellar thickness, and molecular conformation [9,10,16–18]. However, the effect of surface characteristics was barely studied.

Plasma, which is called the fourth state of matter, is a gaseous mixture of neutral atoms and molecules, excited atoms and molecules, radicals and UV photons, and so on. Generally, plasma can be subdivided into thermal plasmas and non-thermal plasmas [19,20]. The surface modifications using plasmas of biodegradable polymers are always exposed to the non-thermal plasmas for less heat degradation in the experiment, which is the weak thermal property of biopolymers [21].

Research of the surface modifications of P(3HB) [22], P(3HB-*co*-3HV) [23–25], and P(3HB-*co*-4HB) [26] has been reported. Pompe et al. showed that fine-tuning the physicochemical surface

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characteristics of P(3HB-*co*-4HB) allows modulation of the availability of adsorbed fibronectin for the formation of cell–matrix adhesions by using the H_2O pressure plasma technique [26]. These results demonstrate that P(3HB-*co*-4HB)-based polymers could be adjusted to a variety of physicochemical characteristics that have utility in medical applications. Wada et al. reported that enzymatic degradability of P(3HB) was decreased by the grafting of acrylic acid after UV irradiation and thermal remolding [22]. However, the enzymatic degradability of the P(3HB-*co*-4HB) copolymers after surface modification has not been reported.

In this paper, a non-thermal plasma was employed for the surface modification of P(3HB) and P(3HB-*co*-4HB) films with poly(-acrylic acid) (PAA–P(3HB) or PAA–P(3HB-*co*-4HB)), and detailed characterization of PAA-grafted PHA was demonstrated. Two kinds of films, solvent-cast films and melt-crystallized films, were degraded with an extracellular PHB depolymerase purified from *Ralstonia pickettii* T1 to control the rate of enzymatic degradability.

2. Experimental

2.1. Materials

Poly[(*R*)-3-hydroxybutyrate-*co*-4.7 mol%-4-hydroxybutyrate] (P(3HB-*co*-4.7 mol%-4HB)) with weight-average molecular weight (M_w) = 5.8×10^5 and polydispersity (DPI) = 2.52 and poly[(*R*)-3-hydroxybutyrate-*co*-9.4 mol%-4-hydroxybutyrate] (P(3HB-*co*-9.4 mol%-4HB)) with $M_w = 3.8 \times 10^5$ and DPI = 1.81 were supplied by Telles LLC Company (Lowell, Massachusetts, USA). All P(3HB-*co*-4HB)s were already purified by Telles LLC Company and used without further purification. Poly[(*R*)-3-hydroxybutyrate] (P(3HB)) with $M_w = 6.4 \times 10^5$ and DPI = 1.89 was supplied by ICI Company. The P(3HB) was purified by re-precipitation in *n*-hexane from chloroform solution and dried under vacuum for 1 week.

Acrylic acid and toluidine blue O were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). The acrylic acid was distilled before use. Poly(acrylic acid) (PAA) with $M_w = 1.0 \times 10^5$ was purchased from Sigma–Aldrich Group (Tokyo, Japan).

2.2. Surface modification with plasma treatment

All grafted polymers were dissolved (0.01 g/mL) in chloroform at room temperature and then heated with solvent at 80 °C for 30 min in a pressure glass tube then cooled to room temperature. Prepared solutions were transferred to 9 cm diameter glass Petri dishes, and kept at room temperature for five days to allow gradual evaporation of the solvent. The melt-crystallized films were prepared from the solvent-cast films, melted at 180 °C for P(3HB-*co*-4HB)s or 200 °C for P(3HB) for 15 s in a hot press at 5 kPa, and then crystallized at 100 °C for 2 h.

The surface modification of films with PAA was performed according to the method reported by Kai et al. [27]. The sample films were inserted into a glass tube and treated at under 10 Pa Ar atmosphere for 60 s by a Radio frequency plasma (RF-Plasma) operating at 13.56 MHz and delivering at 30 W. The films were transferred into acrylic acid aqueous solutions with different acrylic acid content and kept at 40 °C for 20 h. The PAA-grafted P(3HB-*co*-4HB) and P(3HB) films were immersed in water for 2 h and then washed with a large amount of water and ethanol, respectively, to remove any unreacted acrylic acid monomer and PAA homopolymer. The PAA-grafted P(3HB-*co*-4HB) and P(3HB) films were vacuum-dried at room temperature. The grafted films were weighed until constant weight was achieved.

The increase in weight, also known as graft density, was determined from the following equation:

$$\text{Increase in weight} = (W_1 - W_2)/S,$$

where the W_1 and W_2 are the weights of PHA films after and before, respectively, plasma-initiated polymerization. S is the area of the films.

All of these dates were the average of at least 3 times testing.

2.3. Staining with toluidine blue

The toluidine blue O was used to stain the PAA–P(3HB-*co*-4HB) and PAA–P(3HB) films [27]. All films were immersed in an aqueous solution of toluidine blue (0.1 M HCl, 0.034 M NaCl, 0.0013 M toluidine blue O chloride) for 2 h at room temperature. Then, all grafted films were washed with distilled water several times and dried under vacuum.

2.4. Characterization

Molecular weight data were obtained by gel permeation chromatography (GPC) at 40 °C, using a Shimadzu 10A GPC system and a 10A refractive index detector with two linked columns (Shodex K-806M and K-802). Chloroform was used as an eluant at the flow rate of 0.8 mL/min, and 0.5 mg/mL sample solution concentrations were used. Polystyrene standards with low polydispersity were used to produce a molecular weight vs eluant volume calibration curve.

Physical properties of the films (10 mm × 2 mm) were measured at room temperature at a strain rate of 20 mm/min with a Shimadzu EZ-Test (Cap. 500 N) instrument. At least five specimens of each sample were tested.

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) measurements were performed on an FTIR-6100 spectrometer (Jasco, Japan) equipped with an IMV-4000 multichannel infrared microscope (Jasco) with an ATR collector ATR-30-G-45 (Jasco). The PAA sample was tested by blending with the KBr.

Contact angle measurements were performed on a FAMAS DM500 apparatus (Kyowa Interface Science Co. Ltd., Japan) with a 2 μL distilled water droplet on the films at 23 °C and 50% humidity surrounding. At least six specimens of each sample were tested. Scanning electron micrographs (SEMs) were recorded with a Hitachi Ionsubatta E-1030 instrument with Pt, operated at 5 kV acceleration voltage, after samples were coated with iron using an S4000 sputtering device.

All characterization experiments were repeated at least 3 times and the date results were averaged.

2.5. Enzymatic degradation

Extracellular PHB depolymerase purified from *R. pickettii* T1 (previously named *Alcaligenes faecalis* T1) was used for enzymatic degradation of both P(3HB) and P(3HB-*co*-4HB) films. Each 10 mm × 10 mm film sample was incubated with 5 μL of enzyme solution (200 μg/mL) in potassium phosphate buffer (1 mL, pH = 7.4) at 37 °C. Films were periodically removed, washed twice with distilled water, dried to constant weight, and put back into the enzyme solution. Weight losses (average value of at least three specimens) of solvent-cast and melt-crystallized films were recorded over a period of 24 h. Enzymatic degradation was applied to PAA-grafted PHA melt-crystallized films that were divided into four pieces.

3. Results and characterization

In this study, we used three kinds of samples and two kinds of films, each modified with a 1–10% acrylic acid solution (AA). For