

論文の内容の要旨

生物材料科学 専攻

平成 22 年度博士課程 進学

氏名 張 佳奇 (Zhang Jiaqi)

指導教員名 岩田 忠久

論文題目 Structure, physical properties and enzymatic degradation of poly[(*R*)-3-hydroxybutyrate-*co*-4-hydroxybutyrate]
(ポリ[(*R*)-3-ヒドロキシブチレート-*co*-4-ヒドロキシブチレート]の構造、物性及び酵素分解性)

Poly[(*R*)-3-hydroxybutyrate-*co*-4-hydroxybutyrate] (P(3HB-*co*-4HB)) (Fig.1) is the one of polyhydroxyalkanoates (PHAs) which can be produced from renewable resources (such as sugar, plant oil) by the microorganisms and is completely biodegraded into CO₂ and H₂O (Fig.2). Until now, the physical properties and crystal and molecular structures of PHB were studied in depth. Tg and Tm of P(3HB) are 4 °C and 180 °C, respectively. P(3HB) is crystallized as an orthorhombic crystal system with lattice constants of $a = 0.576$ nm, $b = 1.320$ nm and $c(\text{fiber axis}) = 0.596$ nm and P2₁2₁2₁ space group. However, due to secondary crystallization and low elongation to break of P(3HB), it was hard to use for the daily life. Accordingly, PHAs with longer side chains as a second monomer unit are produced by different carbon resources and genetic engineered microorganisms.

In this thesis, P(3HB-*co*-4HB)s with different 4HB contents were used to produce the strong films and fibers by drawing and annealed methods from amorphous state. Furthermore, the plasma modification on the surface of P(3HB-*co*-4HB) films with polyacrylic acid (PAA) was applied to control the enzymatic degradation rate. The polyethylene glycol graft cellulose nano-fibers

(PEG-CF) was offered to increase the crystallization of P(3HB-*co*-4HB).

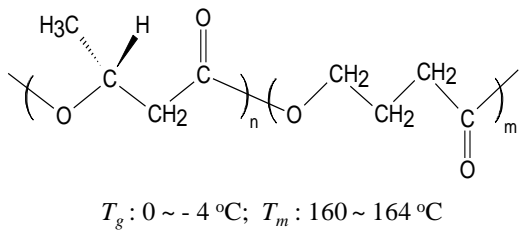


Fig.1 The chemical structure of P(3HB-*co*-4HB)

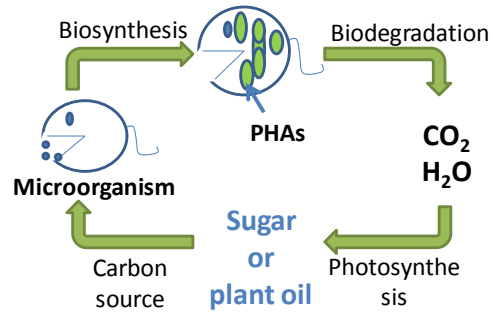


Fig.2 Microbial production and biodegradation of PHB

Chapter 1 introduces the background of PHB biopolymer studies, including the biosynthesis, thermal and physical properties, higher order structure and biodegradation of P(3HB) and its copolymers, were totally introduced.

Chapter 2 describes preparation, mechanical properties and structure analysis of P(3HB-*co*-4HB) films prepared by uniaxial cold-drawing from an amorphous preform at a temperature below, but close to the glass transition temperature (0 °C). Molecular and highly-ordered structure and physical properties of cold-drawn films were investigated by tensile testing, wide-angle X-ray diffraction and small-angle X-ray scattering. Enzymatic degradation of P(3HB-*co*-4HB) films was performed using an extracellular polyhydroxybutyrate depolymerase purified from *Ralstonia pickettii* T1. Tensile strength, elongation to break and Young's modulus of P(3HB-*co*-4HB) with cold-draw ratio 1200% reached 290 MPa, 58% and 2.8 GPa (Fig.3), respectively. X-ray fiber diagrams of 1200% cold-drawn P(3HB-*co*-4.7mol%-4HB) films showed a strong reflection on the equatorial line, indicating a planar zigzag conformation (β -form) together with 2_1 helix conformation (α -form) (Fig.4). The β -form seems to contribute to the high tensile strength, and a new mechanism of generation of the β -form is proposed. The enzymatic degradation rate increased with increase in the draw ratio, and increased greatly with increase in the 4HB content.

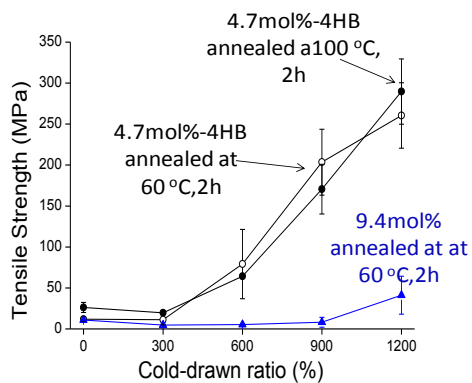


Fig.3 Tensile strength of P(3HB-co-4HB) cold-drawn films

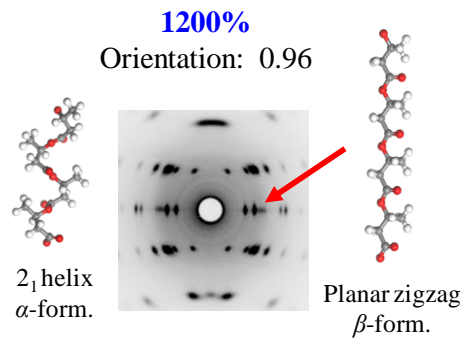


Fig.4 X-ray fiber diagrams of 1200% cold-drawn P(3HB-co-4.7mol%-4HB) films

Chapter 3 presents processing of melt-spun fibers of the P(3HB-co-4HB) with 4.7mol% 4HB contents by spinning at different melting temperatures and take-up speed. Melt-spun fibers were drawn by three different kinds of drawing methods, such as cold-drawing, room-temperature drawing and small-crystal-nuclei drawing. Tensile strength, elongation to break and Young's modulus of P(3HB-co-4HB) fibers (190 °C/100 rpm) with cold-draw ratio of 10 reached 287 MPa, 35% and 5.2 GPa, respectively. On the other hand, small-crystal-nuclei (at 0 °C for 24h) drawn fiber was also shown the similar physical properties. X-ray fiber diagrams of both fibers showed a planar zigzag conformation (β -form) together with 2_1 helix conformation (α -form). The partial enzymatic degradation showed that there is a different highly-ordered structure between small-crystal-nuclei drawing fibers and other fibers.

In Chapter 4, poly(acrylic acid) (PAA) was grafted onto the film surfaces of P(3HB), and two kinds of P(3HB-co-4HB) with 4.7 mol% or 9.4 mol% of 4HB content, by plasma-initiated polymerization. The graft ratio of PAA on the film surface increased drastically with acrylic acid concentration over 8% (Fig. 5). 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 (Fig. 6). 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.

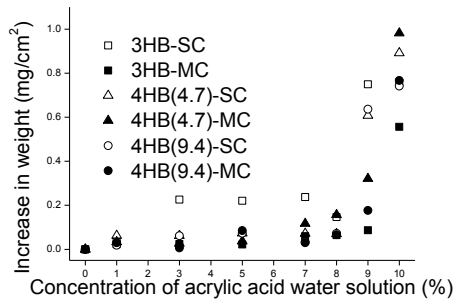


Fig.5 Weight changes of P(3HB-co-4HB) films after grafting with PAA

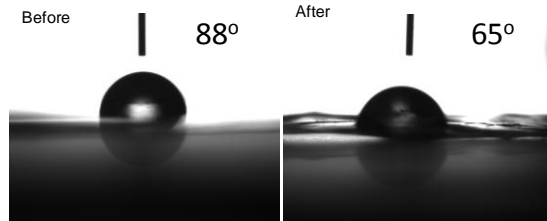


Fig.6 Surface contact angle values of P(3HB-co-4HB) before and after grafting with PAA

Chapter 5 shows the application of polyethylene glycol grafted cellulose nano-fibers (PEG-CF) as bio-based nucleating agents for 3 kinds of P(3HB-co-4HB)s with different 4HB contents (4.7 mol%, 9.4 mol% and 11.0 mol%). Polyethylene glycol (PEG) was also investigated as a nucleating agent to compare the effect on cellulose nano-fibers. The PEG-CF/PHB with the content of 4/96 (w/w) was prepared. The non-isothermal and isothermal crystallization was studied by differential scanning calorimeter (DSC) and polarizing microscope (POM) to assess the crystallization behavior of P(3HB-co-4HB) blending with PEG-CF or PEG. It was shown that the half-time crystallization ($t_{1/2}$) was rapidly decreased by the blend with PEG-CF, indicating that the crystal rate was accelerated (Fig.7). The POM images show that the PEG-CF worked as a nucleating agent for P(3HB-co-4HB) (Fig.8).

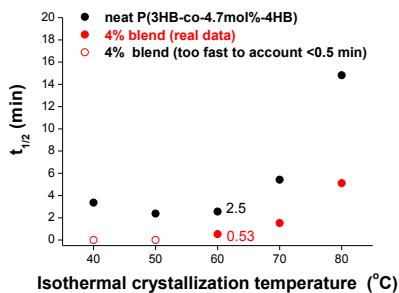


Fig.7 $t_{1/2}$ of isothermal crystallization of neat and blends polymers

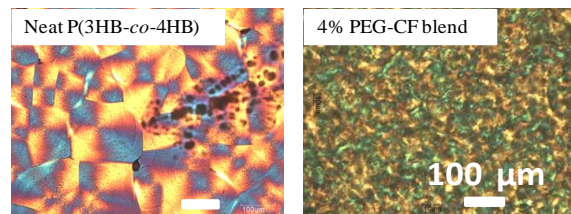


Fig.8 POM images of neat and blend samplers isothermally crystallized at 60°

Chapter 6 provides a summary of the research results presented in this study.