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論 文 要 旨

THESIS SUMMARY

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要旨（英文800語程度）
Thesis Summary（approx.800 English Words ）

Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible aliphatic polyesters synthesized inside the bacterial body. They can be biosynthesized from renewable biomass and carbon resources such as sugars and plant oils and can be biodegraded entirely in the marine environment and designated composting site. Compared to other biodegradable polymers, PHAs are somewhat unique because they can be intercellularly degraded by native depolymerase under a specific condition, and further utilized as intercellular energy storage and carbon source. PHA native producer is known to accumulate mostly P(3HA)s with perfectly straight monomers with a hydroxyl group on their β-carbon, subsequently polymerized by PhaC and resulting in polymers with β-carbon side-chain units. Even though these polymers were vastly studied, the alternative position for a side-chain unit in 3HAs and a variety of side-chain units on 2HAs was not investigated comprehensively. In this study, the biosynthesis of PHAs composed of monomers with α-carbon side-chains is investigated and their material characteristics are evaluated.

In Chapter 1, the general introduction to biomass-based bioplastics and overall PHA material properties are presented.

In Chapter 2, the biosynthesis and characterization of PHA containing 2HA units are presented. The 2HA units are introduced into PHA by the expression of two critical monomer-supplying enzymes extracted from *Peptoclostridium difficile*, namely (*R*)-2-hydroxy-4-methylvalerate (2H4MV) dehydrogenase (LdhA) and 2H4MV-CoA transferase (HadA), along with mutated PHA synthase $PhaC1_{Ps}STQK$ in recombinant *E. coli* DH5α as host strain. 2H4MV is the dominant monomer in the 2HA unit mixture. The precursor leucine and glucose mainly drove the production of 2HAs as carbon sources; such a combination could accumulate 2H4MV units up to 100 mol% at a PHA yield of 13 wt%. Notably, even under the glucose-only feedstock, bacteria can synthesize up 2HAs up to 23.5 mol% in copolymers, indicating the sufficient utilization of structurally related amino acids from cell metabolism by a designed synthetic pathway. A systematic boost in comonomer's composition can be observed when leucine concentration increases from 0.1 to 3 g/L. Furthermore, the material property analysis reveals that when 2HA's molar fraction exceeds 20 mol%, PHA tends to become amorphous with no melting temperature (T_m) and increased glass transition temperature (T_g), where a P(2H4MV) homopolymer exhibits T_g at room temperature. Although PHA with 2HA units over 25 mol% showed modified elongation at break than P(3HB), the low tensile strength value could still be considered a drawback because of its weak durability.

In Chapter 3, the biosynthesis and characterization of PHA containing 3H2MP units are presented. PHA with a single α-carbon methylated unit was accumulated by expressing monomer-supplying enzyme propionyl-CoA transferase (PCT) and PhaC mutant in recombinant *E. coli* LSBJ. Under different plasmid combinations and various culturing conditions, PHAs composed of 3H2MP units are successfully obtained. The (*R*) and (*S*) configurations of the 3H2MP unit can be further introduced as pure enantiomeric precursors, resulting in PHA with distinct tacticity. A highly isotactic P(3H2MP) was reported for the first time under (*S*)-3H2MP only precursor. When the culture medium was fed with (*R*)-3H2MP only precursor, the final polymer exhibited high (*S*)-enantiomer ratio than (*R*)-enantiomer, resulting in an atactic polymer, and the (*R*)-enantiomer's ratio is increased with the incorporated amount of 3HB unit. When compared between 3HB-based copolymers, racemic P(3HB-*co*-3H2MP) with high 3H2MP unit fraction showed much improved

elongation at break while maintaining the same melting temperature to racemic P(3HB-*co*-3H2MP) with relatively low 3H2MP unit fraction. P(3H2MP) homopolymer with different enantiomeric ratios exhibited opposite material properties. Isotactic P(3H2MP) showed high crystallinity and relatively high melting temperature, whereas atactic P(3H2MP) showed complete amorphous characteristics. Additionally, the thermal stability of P(3H2MP) homopolymer samples showed a significantly increased thermal degradation temperature, which is the highest amongst all PHA materials studied.

In Chapter 4, the biosynthesis and characterization of PHA containing 3HPi units are presented. Under the same culturing condition as Chapter 3, P(3HB-*co*-3HPi) copolymers with different 3HPi unit fractions are obtained by adjusting the 3HPi feeding concentration. For 3HB-rich P(3HB-*co*-3HPi), highest 3HPi composition appeared at over 30 mol% when 1 g/L 3HPi acid is added. Under the combination of phasin PhaP and PhaC mutant, all PHA samples exhibited a high weight average molecular weight of over one million g/mol. While the thermal properties of P(3HB-*co*-3HPi) are average, the mechanical performance was outstanding. From tensile test result of P(3HB-*co*-3HPi) samples, the highest elongation at break value was observed, which is also the highest ever reported for biosynthesized PHA. Given its high flexibility, P(3HB-*co*-3HPi) showed elastomeric characteristics where the elongation and recovery are reversible. The mechanism of its elasticity is further confirmed by Wide-angle X-ray diffraction (WAXD) and small-angle X-ray scattering (SAXS). The results of X-ray patterns revealed that during stretching, the randomly distributed crystalline phase and amorphous phase were reformed, and β-form crystals can be observed during force load; upon releasing the stress, such neatly rearranged polymer matrix is back to its initial random state, thus resulting in a reversible rubber-like PHA material.