学 位 論 文 審 査 の 要 旨

博士の専攻分野の名称　博士（総合化学）　氏名　孫 健

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Enzymatic Degradation Mechanism of Lactate-based Polyester Biosynthezied from Biomass Resources
（バイオマスから生合成した乳酸ベースポリエステルの酵素分解メカニズム）

Biodegreadable and bio-based polyesters such as poly(D-3-hydroxybutyrate) [P(3HB)] can be used as environmental friendly plastics to replace non-biodegradable and petrochemical-based plastics which cause plastic pollution and global warming problems. Recently, our group succeed in the biosynthesis of novel copolymer P[D-lactate (LA)-co-D-3HB] [P(LA-co-3HB)] using engineered Escherichia coli expressing lactate-polymerizing enzyme. This copolymer is thought to be a potent bio-based material because of its excellent transparency and flexibility comparing with the parental homopolyesters PDLA and P(3HB). Currently, P(LA-co-3HB) is produced using refined pure sugars such as glucose and xylose from edible biomass, and the biodegradability of this copolymer is unknown. The aim of this study was to explore the possibility of using inedible lignocellulosic biomass as carbon source for P(LA-co-3HB) production and investigate the biodegradability and enzymatic degradation mechanism of P(LA-co-3HB).

In this context, the general introduction including the research theme is described in chapter I.

In chapter II, lignocellulosic biomass-derived hydrolysate was used for biosynthesis of P(LA-co-3HB) which was evaluated in the aspects of cell growth and polymer production. Miscanthus × giganteus was chosen as candidate, because of its high biomass production and low fertilizer requirement at low temperature comparing with other lignocellulosic biomass such as rice straw. After delignification followed by enzymatic hydrolysis, M. × giganteus was converted into mainly glucose and xylose with trace amount of arabinose. Notably, using M. × giganteus-derived hydrolysate, P(LA-co-3HB) was producedat comparable level as the polymer produced from a mixture of pure glucose and xylose. This result indicated that lignocellulosic biomass can be a good feedstock for P(LA-co-3HB) production.

In chapter III, biodegradability of P(67 mol% LA-co-3HB) was investigated by screening and isolation of degrading microorganism from soil samples. Interesingly, half of the samples exhibited degrading activity toward P(67 mol% LA-co-3HB), whereas none of the samples was able to degrade PDLA, suggesting that the biodegradability of P(67 mol% LA-co-3HB) was higher than PDLA. From the soil sample which exhibited the highest degradation activity toward P(67 mol% LA-co-3HB), a P(LA-co-3HB)-degrading microorganism was isolated and identified as Variovorax sp. C34. Further, a P(LA-co-3HB)-degrading enzyme (PhaZVs) was purified from the culture supernatant of Variovorax sp. C34. PhaZVs degraded P(67 mol% LA-co-3HB) and 3HB homopolymer [P(3HB)], but not PDLA. The different degradation behavior of P(LA-co-3HB) and PDLA stimulated the research interest in the mechanism underlying the enzymatic degradation of P(LA-co-3HB) by PhaZVs.

In chapter IV, for study of enzymatic degradation mechanism of P(LA-co-3HB), the cleavage pattern of P(LA-co-3HB) by PhaZVs was investigated. PhaZVs degraded P(LA-co-3HB) into dimers, 3HB-3HB, 3HB-LA, LA-3HB and LA-LA, and monomers, suggesting that PhaZVs cleaved the linkages between LA and 3HB units and between LA units. This cleavage pattern was further proved by PhaZVs conducted hydrolysis of synthetic methyl trimers, 3HB-3HB-3HB, LA-LA-3HB, LA-3HB-LA and 3HB-LA-LA. Combining with the result that PhaZVs did not degrade PDLA homopolymer, the cleavage capability of PhaZVs toward LA-LA linkage should depend on the molecular weight of LA-cluster in the polymer chain. Enzymatic degradation of PDLA oligomers (6 to 40 mer) revealed that the degradation efficiency is inversely proportional to the length of substrates, and PhaZVs is able to degrade oligomers shorter than 31 mer. For the first time the degradation capability of PhaZVs toward PDLA and P(LA-co-3HB) was found to be strongly associated with the molecular weight of LA-cluster containing in these polyesters.

Chapter V is the summary of this study. In short, P(LA-co-3HB) could be produced using lignocellulosic biomass-derived hydrolysate and the enzymatic biodegradation of P(LA-co-3HB) was found that to be dependent on the molecular weight of LA-clustering sequence in the polyester. The trials of this study should contribute to the establishment of a biorefinery using lignocellulosic biomass for production of P(LA-co-3HB) with alterable biodegradability.

Based on the review and interview of the doctor thesis, this study can be judged to be very significant and valuable from the viewpoints of academic research and industrial potential. Ph.D degree of Engineering should be awarded to the candidate.