論文 / 著書情報
Article / Book Information

題目(和文)	クラスIVポリヒドロキシアルカン酸重合酵素のアルコーリシス分解能に関する研究
Title(English)	Study on Alcoholytic Cleavage of Polyhydroxyalkanoate Chains by Class IV Synthases
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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 ）

PHAs are attractive polymeric materials due to not only superior properties such as biodegradability and biocompatibility but also being bio-based polymers. In PHA biosynthesis, PHA synthase catalyzes the polymerization of acyl moiety of 3HA-CoA into PHA. Therefore, PHA synthase is an important enzyme in PHA biosynthesis and a number of studies about PHA synthase have been reported. It is known that the characteristics of polymerized PHA including its composition and molecular weight are varied depending on the types of synthases. Since the material properties of PHA are affected by the capability of employed synthase, expanding our knowledge about PHA synthases are important to develop PHA biosynthesis system.

The present study focuses on class IV synthases which is newly added into the classification. Thus, class IV synthases are expected to possess novel characteristics because these synthases are composed of PhaC as a catalytic subunit and a novel subunit PhaR. In addition, class IV synthases are from genus *Bacillus* which belongs to gram-positive bacteria unlike the well-studied PHA producers belonging to gram-negative bacteria. In fact, it was suggested that one of class IV synthases, $PhaRC_{Bsp}$ from *Bacillus* sp. INT005, possesses a novel PHA degrading activity. However, little is known about this degrading activity. The purpose of this study is to characterize class IV synthases and elucidate the PHA degrading activity of class IV synthases.

In Chapter 2, two class IV synthases from *B. cereus* YB-4 ($PhaRC_{YB4}$) and *B. megaterium* ($PhaRC_{Bm}$) were evaluated by analyzing PHAs synthesized in recombinant *R. eutropha* and *E. coli*. As a result, these two synthases were quite different in PHA production capability and substrate specificity. $PhaRC_{YB4}$ showed higher PHA productivity and broader substrate specificity than $PhaRC_{Bm}$. Also, from the results using *E. coli* as a production host, it was suggested that $PhaRC_{YB4}$ possesses not only PHA polymerization activity but also degradation activity. In contrast, $PhaRC_{YB4}$ did not show PHA degradation activity in *R. eutropha*. Therefore, these results suggested that there might be the regulatory mechanisms governing the PHA degradation activity of $PhaRC_{YB4}$.

In Chapter 3, the mechanism governing the P(3HB) scission activity of $PhaRC_{YB4}$ was investigated. Nuclear magnetic resonance (NMR) analysis revealed that the low-molecular-weight PHA was capped by ethanol at carboxy end. This observation suggests that the molecular weight reduction was the result of alcoholytic cleavage of PHA chains by $PhaRC_{YB4}$ induced by endogenous ethanol. In addition, it was found that the alcoholytic activity was also induced by the presence of exogenous ethanol. Also, the PHA synthase from *Bacillus megaterium* ($PhaRC_{Bm}$) as other class IV synthase was assayed and was shown to have weak alcoholysis activity for PHA chains. These results suggested that class IV synthases might commonly share alcoholysis activity as an inherent feature. In addition, the alcoholytic cleavage activity of $PhaRC_{YB4}$ was detected in vitro. From these observations, it was concluded that P(3HB) cleavage by $PhaRC_{YB4}$ via alcoholysis reaction with endogenous ethanol resulted in the molecular weight decrease.

In Chapter 4, amino acid residue involved in alcoholysis activity and alcohols which could be recognized by $PhaRC_{YB4}$ as substrates for alcoholysis reaction were investigated. Although ester-degrading enzymes generally include lipase-box sequence (Gly-X-Ser-X-Gly) in their amino acid sequence, $PhaRC_{YB4}$ has no lipase-box sequence but has a lipase-box like sequence (Gly-X-Cys-X-Gly). Site-directed mutagenesis on $PhaC_{YB4}$ revealed that three amino acid residues involved in polymerization activity, Cys^{151} in lipase-box like sequence, Asp^{306}, and His^{335} were also involved in alcoholysis activity. In particular, Cys^{151} could be substituted to serine residue for expression of alcoholysis activity. Taking these results together, we proposed the mechanism of alcoholysis reaction by $PhaRC_{YB4}$. As for alcohol preference of $PhaRC_{YB4}$ for alcoholysis reaction, it was found that $PhaRC_{YB4}$ could recognize linear alcohol with 1 to 6 carbon chain and produce end-capped P(3HB) with high modification yield. A variety of alcohols such as poly(ethylene glycol)s, 1,3-propanediol (including hydroxy group), 3-mercapto-1-propanol (thiol group), and 2-propyn-1-propanol (triple bond), benzyl alcohol (benzene ring) could be introduced into P(3HB) carboxy end. These results demonstrated that the alcoholysis reaction with appropriate hydroxy compound would provide the end-capped P(3HB) with desired functional group.

On the basis of the results mentioned above, it can be concluded that class IV synthases have different PHA production capability and substrate specificity depending on its subgroup. As far as we tested, class IV synthases commonly shared novel alcoholysis activity, where three amino acid residues involved in polymerization reaction are also contributed. This alcoholysis reaction could be applied to modify the end structure of PHA and would supply a new strategy for improving the material property and processability of PHA in the future.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).