論文 / 著書情報
Article / Book Information

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Category(English)	Doctoral Thesis
種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

専攻： Department of	物質科学創造　専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　（　工学　）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Polyhydroxyalkanoates (PHAs) are aliphatic polyesters synthesized by a variety of microorganisms as intracellular carbon and energy storage. PHAs are expected to be applied as sustainable plastics, which can be synthesized from renewable biomass such as sugars, fatty acids, and plant oils. PHA polymerization reaction is catalyzed by PHA synthase (generally named PhaC) and this enzyme is a one of the most important proteins in PHA biosynthesis. This thesis focused on PhaC's behavior by investigating the effect of amphiphilic molecules, which were nonionic detergents and PHA binding protein (named phasin or PhaP), on PhaC reactivity.

In Chapter 1, general introduction about PHA and its biosynthesis systems were described. Additionally, the reaction mechanisms and reaction properties of PHA synthase, particularly *in vitro* (extracellular) reaction, were reviewed.

In Chapter 2, the effect of nonionic detergents on reaction property of PHA synthase from *Ralstonia eutropha* ($PhaC_{Re}$) was evaluated by *in vitro* experiments. A wide variety of nonionic detergents, especially TritonX-100, eliminated lag phase and activated $PhaC_{Re}$ at sub-critical micelle concentration (sub-CMC). Additionally, an application a nonionic detergent for kinetic analysis of $PhaC_{Re}$ was demonstrated. This kinetic analysis suggests that 3'-phosphate group in CoA moiety would be important to make a stable enzyme-substrate complex, providing new insight into recognition mechanism of CoA moiety by $PhaC_{Re}$.

In Chapter 3, a PhaC activator working in *in vivo* was explored. Phasin (PhaP) is known as a PHA granule binding protein in native PHA producers and it is amphiphilic protein containing hydrophobic and hydrophilic regions to bind PHA surface and dissolve in water, respectively. Thus, PhaP can be regarded as detergent-like molecule. Based on the functional similarity of PhaP with nonionic detergents, PhaP was tested as an activator of PhaC in both *in vitro* and *in vivo* experiments. PhaPs from *Aeromonas caviae* ($PhaP_{Ac}$) and *R. eutropha* ($PhaP1_{Re}$) were added into *in vitro* reaction mixture containing the synthesized substrate with PhaC from *A. caviae* ($PhaC_{Ac}$), *R. eutropha* ($PhaC_{Re}$), or *Delftia acidovorans* ($PhaC_{Da}$). The activity assays exhibited an activation effect of both PhaPs for $PhaC_{Ac}$ but inhibitory effect for $PhaC_{Re}$ and $PhaC_{Da}$ depending on PhaP concentrations. PhaPs increased not only activity of $PhaC_{Ac}$ but also affinity for C6 monomer unit. The modulation effect of PhaPs for PhaC was evaluated *in vivo*. PHA biosynthesis from glucose in the recombinants showed enhanced PHA accumulation in both $PhaC_{Ac}$ and $PhaC_{Re}$ strains with increasing PhaP expression. To understand the difference in $PhaC_{Re}$ activity between *in vitro* and *in vivo*, the PhaP-dependency of PhaC activity was investigated *in vivo*. The result suggested that $PhaP_{Ac}$ solubilized $PhaC_{Re}$ just like chaperons, thereby increasing the amount of active $PhaC_{Re}$. Molecular weights analysis for the accumulated PHA also supported the predicted function of $PhaP_{Ac}$. Therefore, PhaPs gave a positive effect on PHA accumulation depending on PhaP concentrations due to another function as an activator or a chaperon-like protein.

In Chapter 4, the effect of a single base mutation (G → A) in the 10^{th} nucleotide of phasin gene ($phaP_{Ac}$) on PHA accumulation was investigated. The single base mutation was introduced into *phaPCJ*-expressing and *phaP*-expressing plasmids. In both of recombinants harboring these plasmids, the single base mutation in $phaP_{Ac}$ gene showed improved PHA accumulation. Analyses of protein expression levels indicated that the expression level of $phaP_{Ac}$ and its downstream genes were elevated by the single nucleotide mutation in $phaP_{Ac}$ gene. Additionally, *in vitro* transcription was performed to understand the mechanism of the improved expression of $phaP_{Ac}$ by the single nucleotide mutation. From the results of *in vitro* transcription and some studies, the mechanism of the improved expression of $phaP_{Ac}$ was speculated that the nucleotide substitution from G to A in N-terminal region caused the structural change of mRNA, resulting in an efficient transcription of *phaP* gene. This finding provides an important clue to improve the expression efficiency of $PhaP_{Ac}$, leading to an efficient production of PHA.

In conclusion, this thesis documents the function of amphiphilic molecules, nonionic detergents and PhaPs, as an activity modulator of PhaC by *in vitro* and *in vivo* assays. Furthermore, this thesis documents a new function of PhaPs and improved PHA production by enhanced expression of PhaPs. Based on the findings in this study, a $PhaP_{Ac}$-overexpressing mutant was constructed for efficient PHA production. The knowledge about the function of amphiphilic molecules as activity modulator of PhaC would help to understand the reaction mechanism of PhaC and synthesize PHA efficiently.

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).