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Phosphotransacetylase as a Key Factor in Biological Production of Polyhydroxybutyrate

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Abstract

Phosphotransacetylase (Pta) catalyzes the reversible conversion of acetyl-coenzyme A (CoA) to acetyl phosphate. Polyhydroxybutyrate (PHB) synthase and accumulation were compared between a Pta-deficient mutant and the wild-type *Escherichia coli*, which were transformed with pAE100, coding for 3-ketothiolase, NADPH-dependent acetoacetyl-CoA reductase, and PHB synthase from *Ralstonia eutropha*. During the growth period, PHB synthase activity in the Pta-deficient mutant was lower than that in the wild type. PHB accumulation in the Pta-deficient mutant, however, was higher than that in wild-type cells grown in Luria-Bertani (LB) medium containing 1% glucose (high C:N ratio). The Pta-deficient mutant showed PHB accumulation even in LB medium (low C:N ratio), whereas wild-type cells showed no PHB accumulation. These data suggest the activation of PHB synthase by acetyl phosphate that is synthesized by Pta. A decrease in Pta activity probably causes some increase in acetyl-CoA as substrate for the PHB synthesis pathway, resulting in increased PHB accumulation.

Index Entries: Polyhydroxybutyrate; cyanobacteria; phosphotransacetylase; metabolic engineering; pta; ack.

Introduction

Polyhydroxyalkanoates (PHAs) are considered to be strong candidates for biodegradable polymer material because they possess material

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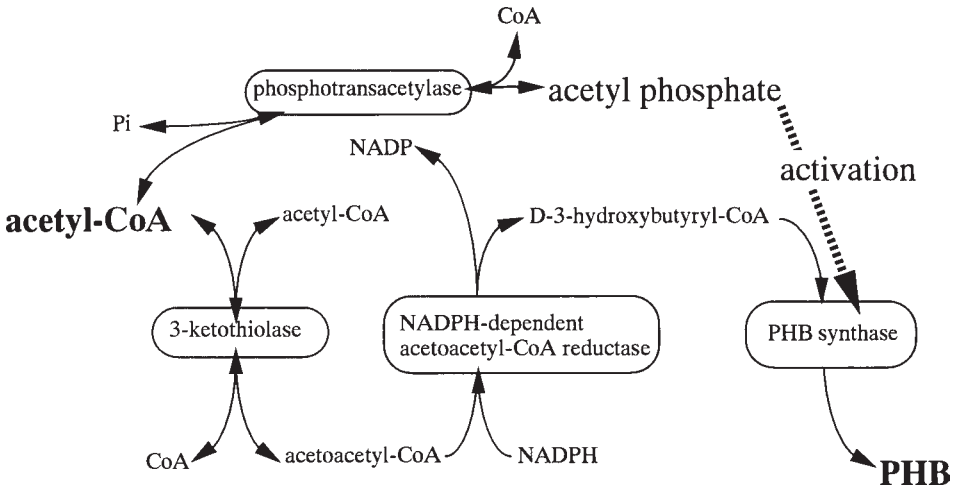

Fig. 1. Model of the regulation of PHB accumulation mediated by acetyl phosphate.

properties similar to those of various synthetic thermoplastics and elastomers currently in use. Many bacterial species have been reported to accumulate PHA *(1)*.

Polyhydroxybutyrate (PHB) is synthesized from acetyl-coenzyme A (CoA) via three enzymatic reactions *(2)*: 3-ketothiolase converts two acetyl-CoA molecules to one acetoacetyl-CoA molecule; acetoacetyl-CoA reductase converts acetoacetyl-CoA to D-3-hydroxybutyryl-CoA with NADPH oxidation; and the last enzyme, PHB synthase, catalyzes the linking of the D-3-hydroxybutyryl moiety to an existing PHB molecule by an ester bond. Possible limiting steps of PHA synthesis are enzyme activity, supply of NAD(P)H, and intracellular acetyl-CoA concentration. It has been shown that the development of recombinant *Escherichia coli* strains harboring a high copy number plasmid carrying PHA biosynthesis genes *(3)* improves PHA productivity.

In some cases, PHB synthase activity regulates PHB accumulation. The cyanobacterium *Synechococcus* sp. MA19 accumulates PHB under nitrogen-deprived conditions *(4)*, owing to increasing activity of PHB synthase in this strain. Recently, it was demonstrated that PHB synthase, which is extracted from *Synechococcus* sp. MA19 cells grown in nitrogen-sufficient conditions, was activated by acetyl phosphate in vitro *(5)*. The intracellular activity of phosphotransacetylase (Pta), which catalyzes the conversion of acetyl-CoA to acetyl phosphate, increases under nitrogen-deprived conditions *(5)*. Under these conditions, the intracellular activity of Pta, which catalyzes the conversion of acetyl-CoA to acetyl phosphate, increases significantly, which points toward an essential role of Pta in the regulation of PHB synthase (Fig. 1).

In this article, we report on an investigation on the biological role of Pta in genetically altered *E. coli*, harboring the PHB synthesis enzymes

Table 1
Bacterial Strains and Plasmids

Bacteria/plasmid	Genotype	Reference
E. coli JM103	Δ*(lac-pro) thi strA supE endA sbcB hsdR/F'traD36 proAB lacIqZ ΔM15*	*8*
E. coli KH131	JM103, Δ*ackA-pta*, Cm®	*6*
JM103 (pAE100)	*E. coli* JM103 harboring pAE100 Amp®	This work
KH131 (pAE100)	*E. coli* KH131 harboring pAE100 Cm®, Amp®	This work
pUC18	*lacZ'*, Amp®	*8*
pAE100	pUC18, *R. eutropha phaC*, *phaA*, *phaB*, Amp®	*9*

encoding genes from *Ralstonia eutropha*. The results, derived from in vitro and in vivo experiments, have led to the proposal of a model (Fig. 1) describing the acetyl phosphate-mediated regulation of PHB synthase.

Materials and Methods

Bacterial Strain, Plasmids, and Growth Conditions

Table 1 gives the bacterial strains and the plasmids. *E. coli* strain KH131 *(6)*, which lacks *pta* gene by homologous recombination of *cat* gene, was kindly gifted by Professor Ichihara, Department of Agriculture of Nagoya University. *E. coli* strains were grown in Luria-Bertani medium at 37°C on a shaker at 50 rpm unless otherwise stated. The mutant strain, KH131 *(6)*, was grown in the presence of 30 μg/mL of chloramphenicol. For stable cultivation of the transformants harboring pAE100, 50 μg/mL of ampicillin was added to the culture medium.

Genetic Manipulation

Transformation of *E. coil* JM103 and KH131 with pAE100 (Fig. 2) was performed by electroporation *(7)*. The selection of the transformants and maintenance were carried out in the presence of 50 μg/mL of ampicillin.

Enzyme Assay

PHB synthase and Pta activities were determined photospectroscopically based on the reaction between CoA and 5,5'-dithiobis(2-nitrobenzoic acid) *(5)*. The β-galactosidase assay was carried out according to standard procedure *(8)*.

Determination of PHB Content

PHB content was determined using a gas chromatograph GC-14A (Shimadzu, Kyoto, Japan) as described by Miyake et al. *(4)*.

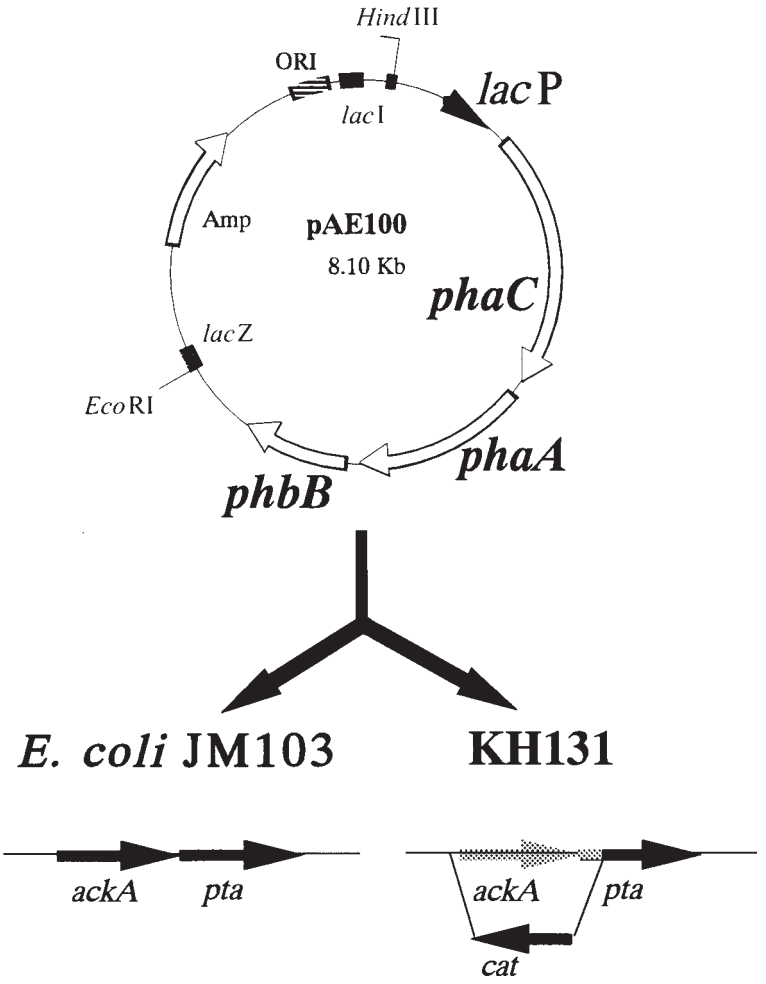

Fig. 2. Transformation of *E. coli* JM103 and KH131 with the plasmid pAE100. *phaC*, PHB synthase; *phaA*, 3-ketothiolase; *phaB*, NADPH-dependent acetoacetyl-CoA reductase; *ackA*, acetate kinase A; *pta*, phosphotransacetylase.

Results and Discussion

Effect of Pta-Acetate Kinase A on PHB Synthase

E. coli, which originally had no PHB accumulation capability, was used to investigate the effect of the Pta-acetate kinase A (AckA) system on PHB synthase. The plasmid pAE100 was incorporated into both of the strains, JM103 and KH131. PHB synthesis genes (*phaC*, *phaA*, and *phaB*) of this plasmid were expressed under *lac* promoter control *(9)*. Figure 3 shows the growth and enzymatic profiles of the transformants. The KH131 (pAE100) was grown slowly owing to the lack of the *pta-ackA* system compared to JM103 (pAE100). In the logarithmic growth phase, KH131 (pAE100) retained lower Pta activity than JM103 (pAE100). Interestingly,

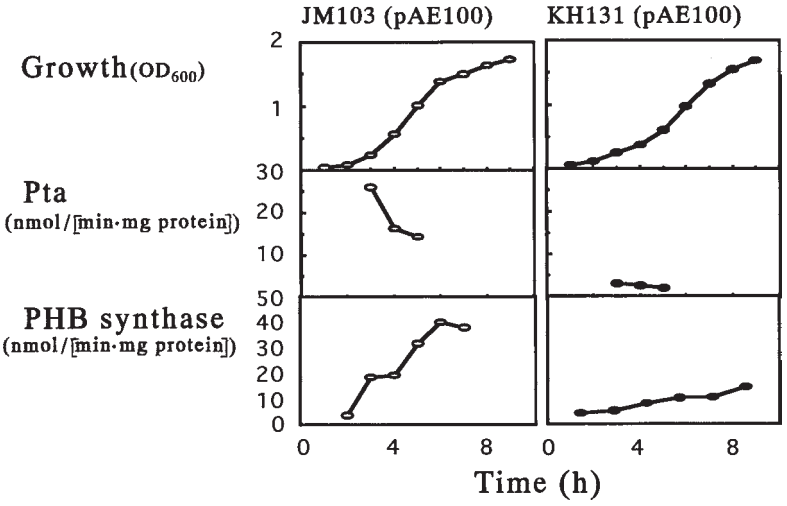

Fig. 3. Pta and PHB synthase activities in transformed *E. coli* harboring the plasmid pAE100.

the PHB synthase activity in KH131 (pAE100) was lower than that in JM103 (pAE100) throughout the growth period. However, β-galactosidase assays of the transformants harboring pUC18 indicated no difference in the *lac* promoter activity between the strains. These results suggest that the Pta-AckA system affects the PHB synthase activity. The lack of Pta-AckA probably results in a decrease in acetyl phosphate. In the cyanobacterium *Synechococcus* sp. MA19, PHB synthase is activated by acetyl phosphate in vitro *(5)*. These results suggest that the lower activity of PHB synthase in KH131 (pAE100) supported the activation of PHB synthase in vivo.

Effect of Pta-AckA on PHB Accumulation

Additionally, the impact of the absence of Pta-AckA on PHB accumulation was investigated (Fig. 4). The KH131 (pAE100) was able to accumulate PHB under a low C:N ratio whereas JM103 (pAE100) showed no accumulation of PHB at all. Under a high C:N ratio, the KH131 (pAE100) demonstrated higher PHB accumulation than JM103 (pAE100). Although KH131 (pAE100) showed lower PHB synthase activity (Fig. 3), PHB accumulation by KH131 (pAE100) was higher than that of JM103 (pAE100). From these data, it can be concluded that the decrease in Pta activity causes an increase in acetyl-CoA as substrate for the PHB synthesis pathway, resulting in increased PHB accumulation. This suggestion is further substantiated by other investigations on a cyanobacterial insertion mutant demonstrating a significant decrease in Pta activity. This mutant enhanced PHB accumulation, probably owing to increased acetyl-CoA concentration in vivo *(10)*. These results lead to the conclusion that in the absence of acetate as carbon source in the culture medium, Pta activity is very likely to affect PHB synthase and PHB accumulation, rather than AckA.

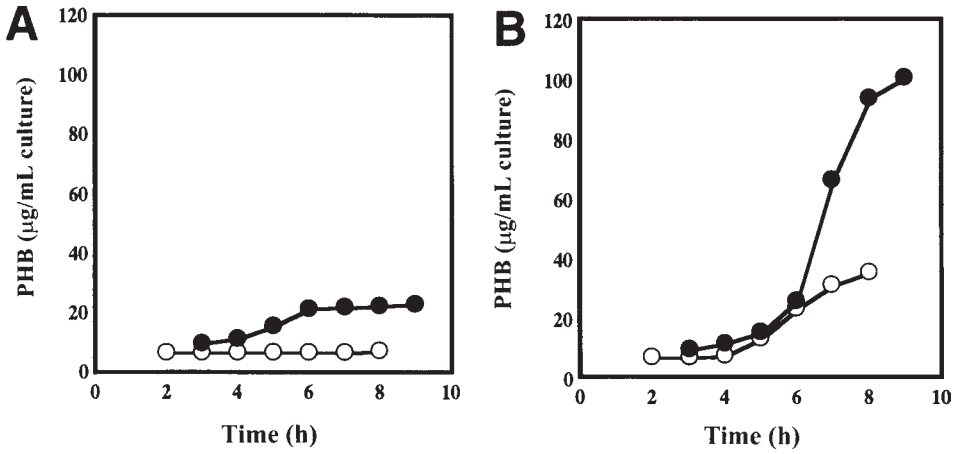

Fig. 4. PHB accumulation in transformed *E. coli* harboring pAE100. **(A)** Strains were grown in LB medium (low C:N ratio). **(B)** Strains were grown in LB medium containing 1% glucose (high C:N ratio). (○) and (●), JM103 (pAE100) and KH131 (pAE100), respectively.

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