I-1. DIVERSE ENZYMES ACTING ON 3-HYDROXYBUTYRIC ACID ESTERS

Terumi Saito
Department of Biological Sciences, Faculty of Science, Kanagawa University
2946 Tsuchiya, Hiratsuka, Kanagawa, 259-1293 Japan.

INTRODUCTION

In poly(3-hydroxybutyrate) (PHB) degradative metabolism, recent studies show that several hydrolases play roles. They may be divided into two major classes: intracelluar enzymes and extracellular enzymes. According to the other classification, these enzymes may fall into PHA depolymerase, PHB depolymerase and 3-hydroxybutyrate (3HB) oligomer hydrolase. From the mode of action, these enzymes can be divided into exo-type and endo-type. The diversity of these enzymes was studied.

MATERIALS AND METHODS

In the case of PHB degrading bacteria, bacteria were grown in a salt medium containing PHB as sole carbon. *Ralstonia eutropha* was grown in a nutrient-rich medium. To produce PHB, cells grown in a nutrient-rich medium were transferred to a nitrogen-free medium containing 2% fructose. *E. coli* JM109 and S17-1/λpir were used as a cloning and expression host, respectively. DNA manipulation was carried out according to standard techniques.

RESULTS

Extracellular PHB depolymerase: Many studies have clarified the basic structure of enzymes in this class [1]. They are composed of three different parts – catalytic domain with a typical lipase box, interdomain part, and substrate binding domain. From bacteria degrading polypropiolactone, a depolymerase was purified and its structural gene was examined. This enzyme was found to belong to the class of PHB depolymerase that has a conserved amino acid sequence, Gly-X_1-Ser-X_2-Gly (lipase box) at the N-terminus [2]. On the other hand, an extracellular PHB depolymerase from *Penicillium funiculosum* was purified. The enzyme has a molecular mass of 37,000 Da with N-terminus, TALPAFNVNPNSVSVSGLSSGGYMAAQL, in which a lipase box sequence was found. This enzyme showed much higher 3HB dimer-hydrolyzing activity than PHB-degrading activity compared with typical bacterial extracellular PHB depolymerases. From nucleotide sequence from genomic library this enzyme seems to lack the substrate binding domain, which was supported by PHB-binding assay. This is a new type of extracellular PHB depolymerase.

Extracellular 3HB oligomer hydrolase: A few PHB-degrading bacteria secretes 3HB oligomer hydrolase along with PHB depolymerase. Two similar enzymes have been isolated from *R. pickettii* strain T1 [3] and A1[4]. They have rather large molecular mass (about 80 kDa). Analysis of the structural gene revealed that there is no classical lipase box in it [4].

Intracellular PHB depolymerase: Recently, an intracellular PHB depolymerase gene was cloned from *Ralstonia eutropha* H16 [5]. The gene product degrades amorphous PHB granules, but not crystalline ones. The predicted molecular mass was about 47 kDa. Most of the product from enzymatic digestion of PHB was oligomers. In the deduced amino acid sequence, there was no typical lipase box sequence. In the sequence there is a conserved region containing a cysteine residue, which may have a important role in catalysis. The gene product was expressed in *R. eutropha* cells concomitant with the synthesis of PHB and localized solely in PHB granules. Although a mutant of *R. eutropha* whose *phaZ* gene was disrupted showed a higher PHB content compared to the wild type in a nutrient-rich medium, it accumulated PHB as much as the wild type did in a nitrogen-free medium. These results indicate that the degradation system in *R. eutropha* is rather complicated. A similar conclusion was obtained by others [6]. It has been reported that another PHB depolymerase exists in the supernatant fraction of *R. eutropha* [7].

Intracellular 3HB-oligomer hydrolase: There are two types of intracellular 3HB-oligomer hydrolases. One is high-molecular mass enzyme (equivalent to the extracellular 3HB-oligomer hydrolase) and the other is low-molecular mass enzyme (about 30 kDa). The gene of the former enzyme was cloned from *R. eutropha*, and the gene of the latter enzyme was cloned from a PHB-degrading bacterium, *Pseudonomas* sp. strain Sa1. The enzyme with high-molecular mass had not a conserved lipase box sequence, but the enzyme with low-molecular mass had it.

CONCLUSIONS

The above overview shows us that the enzymes acting on 3HB esters are various and may be derived from different ancestors, although they all degrade the ester bond formed by 3-hydroxybutyric acid.

REFERENCES

[1] D. Jendrossek, B, Müller, H. G. Schlegel: *Appl. Microbiol. Biotechnol.* **46**, 451-463 (1996)

[2] K. Kobayashi, S. Sugiyama, Y. Kawase, T. Saito, J. Mergaert, J. Swing: *J. Envron. Polym. Degrad.* 7, 9-18 (1999)

[3] Y. Shirakura, T. Fukui, T. Tanio, K. Nakayama, R. Matsuno, K. Tomita: *Biochim. Biophys. Acta* **748**, 331-339 (1983)

[4] K. Zhang, M. Shiraki, T. Saito: *J. Bacteriol.* **179**, 72-77 (1997)

[5] H. Saegusa, M. Shiraki, C. Kanai, T. Saito: *J. Bacteriol.* **183**, 94-100 (2001)

[6] R. Handrick, S. Reinhardt, D. Jendrossek: J. Bacteriol. **182**, 5916-5918 (2000)

[7] T. Saito, K. Takizawa, H. Saegusa: *Can. J. Microbiol.* **41**, 187-191 (1995)