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Synthesis and properties of biodegradable polymers and plastics

Y. Doi

Polymer Chemistry Laboratory, The Institute of Physical and Chemical Research (RIKEN),
Hirosawa, Wako-shi, Saitama 351-01, Japan

Three types of copolymers, poly(3-hydoxybutyrate-co-3-hydroxyvalerate), poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxypropionate), are produced by *Alcaligenes eutrophus* from various carbon substrates. These microbial polyesters are thermoplastics with biodegradable properties, and the physical properties can be regulated by varying the compositions of copolymers.

1. INTRODUCTION

A wide variety of microorganisms accumulate an optically active polymer of (R)-3-hydroxybutyric acid, P(3HB),as an intracellular storage material of carbon and enegy (Anderson and Dawes, 1990; Doi, 1990). Many prokaryotic organisms such as bacteria and cyanobacteria have been found to accumulate P(3HB) up to 80% of their cellular dry weight, when growth is limited by the depletion of an essential nutrient such as nitrogen, oxygen, phosphorus or magnesium. Recently, some bacteria have been found to accumulate copolymers containing (R)-3-hydroxyalkanoate units other than (R)-3-hydroxybutyrate. More recently, copolymers containing 4-hydroxybutyrate unit have been produced by several bacterial strains. The general class of microbial polyesters is called poly (hydroxyalkanoates) (PHA)(Anderson and Dawes, 1990; Doi, 1990).

These microbial polyesters have attracted much attention as environmentally degradable thermoplastics for a wide range of agricultural, marine and medical applications (Holmes, 1988). The microbial polyesters are degradable in soil, sludge or sea water. Some microorganisms such as bacteria and fungi secrete extracellular P(3HB) depolymerase to degrade environmental microbial polyesters and utilize the decomposed compounds as nutrient.

This paper surveys the PHA copolymers produced by *Alcaligenes eutrophus* from various carbon sources and discusses the pathway and its regulation of PHA synthesis. In addition, the biodegradability of PHA products is studied.

2. PRODUCTION OF P(3HB-co-3HV) COPOLYMER

The copolymers of (R)-3-hydroxybutyrate and (R)-3-hydroxyvalerate P(3HB-co-3HV) have been commercially produced by ICI,UK in a large scale, two-stage, fed-batch fermentation of *A.eutrophus*, feeding propionic acid and glucose as the carbon sources (Byrom, 1987). In the first stage, *A.eutrophus* cells grow and multiply in a glucose-salts medium under conditions of carbon and nutrient excess. In the second stage, the phosphate supply becomes depleted and propionic acid is fed. The P(3HB-co-3HV) copolymers are accumulated up to 75% of total dry cell weight in the second stage of phosphate limitation. The total fermentation time is in the order of 110-120 hours. The copolyester compositions vary from 0 to 47 mol% 3HV, depending on the ratio of propionic acid and glucose supplied. The biosynthetic pathway of P(3HB-co-3HV) in *A.eutrophus* has been investigated by using ^{13}C-labeled acetic and propionic acids as the carbon sources (Doi et al., 1987).

The P(3HB-co-3HV) copolymers with a wide range of compositions from 0 to 90 mol% 3HV are accumulated in *A.eutrophus* by using butyric and pentanoic acids as the carbon sources (Doi et

Table 1

Physical and mechanical properties of P(3HB-co-4HB) films at 23°C

Composition(mol %)		Crystallinity (%)	Density (g/cm^3)	Stress at yield (MPa)	Elongation at yield (%)	Tensile strength (MPa)	Elongation to break (%)
3HB	4HB						
100	0	60±5	1.250	-	-	43	5
97	3	55±5	n.d.	34	4	28	45
90	10	45±5	1.232	28	5	24	242
84	16	45±5	1.234	19	7	26	444
56	44	15±5	n.d.	-	-	10	511

al.,1988). The butyric and pentanoic acids are respectively incorporated into 3HB and 3HV units via acetoacetyl-CoA in the β–oxidation cycle. The regulation of P(3HB-co-3HV) biosynthesis from butyric and pentanoic acids is of interest because the key regulatory enzyme of polymer synthesis from acetyl-CoA, 3-ketothiolase, is not involved.

The P(3HB-co-3HV) copolymers were produced at 30°C from various carbon sources by *A.eutrophus* under fed-batch growth conditions. The production of P(3HB-co-3HV) from butyric and pentanoic acids was effective under nitrogen-limited conditions, and the conversion of carbon sources into copolymers was as high as 56 wt% at the C/N mole ratio of 40. In contrast, under nitrogen-excess conditions (C/N<10), the cell growth was good, while the production of P(3HB-co-3HV) from fructose and propionic acid was almost completely inhibited under nitrogen-excess conditions.

3. PRODUCTION OF P(3HB-co-4HB) COPOLYMER

The copolymers of (R)-3-hydroxybutyrate and 4-hydroxybutyrate P(3HB-co-4HB) are produced by *A.eutrophus* from 4-hydroxybutyric acid, 1,4-butanediol or γ–butyrolactone (Kunioka et al., 1988; 1989). When 4-hydroxybutyric acid was used as the sole carbon source, a P(3HB-co-33%4HB) was produced. The addition of butyric acid in the 4-hydroxybutyric acid culture solution resulted in a decrease in the 4HB fraction. Thus, the copolymer compositions were varied from 0 to 33 mol% 4HB, depending on the carbon substrates supplied in the feed. Recently, we have found that the P(3HB-co-4HB) copolymers with a wide range of compositions from 0 to 100 mol% 4HB are produced by *A.eutrophus* from 4-hydroxybutyric acid in the presence of some additives. When 4- hydroxybutyric acid, citrate and ammonium sulfate were fed as the mixed substrates, P(3HB-co-4HB) copolymers with compositions of 70-100 mol% 4HB were produced.

4-Hydroxybutyryl-CoA is first formed from 4-hydroxybutyric acid in the *A.eutrophus* cells. A portion of 4-hydroxybutyryl-CoA is then metabolized into (R)-3-hydroxybutyryl-CoA via acetoacetyl-CoA in the β–oxidation cycle. A random copolymer of 3HB and 4HB units is synthesized by the copolymerization of (R)-3-hydroxybutyryl-CoA with 4-hydroxybutyryl-CoA under the actions of P(3HB) polymerase. When $(NH_4)_2SO_4$ and citrate are added to *A.eutrophus*, acetoacetyl-CoA from 4-hydroxybutyryl-CoA is metabolized into acetyl-CoA rather than into (R)-3-hydroxybutyryl-CoA under growth conditions, resulting in an increase in the 4HB fraction.

The copolymers of (R)-3-hydroxybutyrate and 3-hydroxypropionate P(3HB-co-3HP) are produced by *A.eutrophus*, when 3-hydroxypropionic acid is used as the carbon source (Nakamura et al., 1991). The 3HP content is still limited to the range 0-7 mol%. The P(3HB-co-3HP) copolymers were also produced

from the alkanediols of odd carbon numbers such as 1,5-pentanediol, 1,7-heptanediol and 1,9-nonanediol. In contrast, P(3HB-co-4HB) copolymers were produced from the alkanediols of even carbon numbers such as 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol and 1,12-dodecanediol.

These microbial polyesters are thermoplastics with biodegradable properties (Kunioka et al., 1989; Scandola et al., 1990), and the mechanical properties can be regulated by varying the compositions of copolymers (Table 1).

4. BIODEGRADATION OF MICROBIAL POLYESTERS

A remarkable characteristic of microbial polyesters is that they are thermoplastic with environmentally degradable properties. The biodegradability of PHA products has been studied in environments such as soil, aerobic sewage and sea water. The processes of biodegradation were analyzed by monitoring the time-dependent changes in weight loss (erosion), molecular weights and mechanical strength of films, plates and fibers of microbial polyesters. All the samples exposed in environments were degraded via surface erosion. The rates of surface erosion of P(3HB) film in various environments at 25 °C are given in Table 2. The rate of surface erosion in sea water was almost independent of the copolymer compositions of P(3HB-co-3HV) and P(3HB-co-4HB) samples. In contrast, the erosion rates in soil and aerobic sewage were strongly dependent on copolymer compositions and decreased in the order P(3HB-co-4HB)>P(3HB)>P(3HB-co-3HV). These results suggest that extracellular P(3HB) depolymerases from various bacteria have different specificities on the degradation of microbial polyesters.

An extracellular P(3HB) depolymerase was purified from *Alcaligenes faecalis* which had been isolated in aerobic sewage (Tanio et al., 1982). In a previous paper (Doi et al., 1990), we showed that the rate of enzymatic degradation of PHA films was faster by two or three orders of magnitude than the rate of simple hydrolytic degradation.

Table 2
Decrease in the thickness of P(3HB) films in various environments at 25°C

Environment	Rate of degradation (μm/week)
Aerobic sewage	7
Soil	5
Sea water	5

The enzymatic degradation occurred at the surface of PHA film and the rate of surface erosion decreased in the order P(3HB-co-4HB) > P(3HB) > P(3HB-co-3HV).

REFERENCES

Anderson, A.J.; Dawes, E.A. *Microbiol. Rev.* **1990**, *54*, 450-472.

Byrom, D. *Trends Biotechnol.* **1987**, *5*, 246-250.

Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. *Macromolecules* **1987**, *20*, 2988-2991.

Doi, Y.; Tamaki, A.; Kunioka, M.; Soga, K.;*Appl. Microbiol. Biotechnol.* **1988**, *28*, 330-334.

Doi, Y.; Kanesawa, Y.; Kunioka, M. *Macromolecules* **1990**, *23*, 26-31.

Doi, Y. *Microbial Polyesters*; VCH Pub., New York, **1990**.

Holmes, P.A. In *Development in Crystalline Polymers-2*; Bassett, D.C. Ed.; Elsevier, London, **1988**, pp 1-65.

Kunioka, M.; Nakamura, Y.; Doi, Y. *Polym. Commun.* **1988**, *29*, 174-176.

Kunioka, M.; Kawaguchi, Y.; Doi, Y. *Appl. Microbiol. Biotechnol.* **1989**, *30*, 569-573.

Kunioka, M.; Tamaki, A.; Doi, Y. *Macromolecules* **1989**, *22*, 694-697.

Nakamura, S.; Kunioka, M.; Doi, Y. *Macromol, Rep.* **1991**, *A28*, 15-24.

Scandola, M.; Ceccoruli, G.; Doi, Y. *Int. J. Biol. Macromol.* **1990**, *12*, 112-117.
Tanio, T.; Fukui, T.; Shirakura, Y.; Saito, T.; Tomita, K.; Kaiho, T.; Masamune, S. *Eur. J. Biochem.* **1982**, *124*, 71-77.