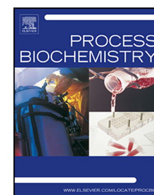
Process Biochemistry 51 (2016) 950–958

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio



Molecular mass of poly-3-hydroxybutyrate (P3HB) produced by *Azotobacter vinelandii* is determined by the ratio of synthesis and degradation under fixed dissolved oxygen tension

Modesto Millán [a], Daniel Segura [b], Enrique Galindo [a], Carlos Peña [a,*]

[a] Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, UNAM, México
[b] Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM, México

ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 31 March 2016
Accepted 16 April 2016
Available online 21 April 2016

Keywords:
P3HB
Molecular mass
A. vinelandii

ABSTRACT

Poly-3-hydroxybutyrate (P3HB) is an intracellular polyester produced by numerous bacteria, including *Azotobacter vinelandii*. Thermo-mechanical properties and biomedical applications of P3HB depend on its molecular mass (MM), which in turn is controlled by the balance between synthesis and degradation of the polymer during its biosynthesis. The aim of this study was to determine the activity levels of enzymes involved in the synthesis and degradation of P3HB and their effect on the molecular mass of the polymer produced by *A. vinelandii* strain OP under the conditions of dissolved oxygen tension of 1 and 15%. The results show that the MM of P3HB changed between the exponential and stationary growth phases, under both oxygen conditions. During the exponential growth phase, the mean molecular mass (MMM) of P3HB was high (4800 kDa), coincident with a high activity P3HB synthase and with a low activity of P3HB depolymerase. In contrast, during the stationary phase, the P3HB MM decreased to 3600 kDa, because of the increased activity of the P3HB depolymerase.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Poly-3-hydroxybutyrate (P3HB) is a homopolymer of 3-hydroxybutyrate that belongs to the family of polyhydroxyalkanoates (PHAs), accumulated as carbon and energy storage material, forming intracellular granules in several bacteria, including *Azotobacter vinelandii* [1]. P3HB has some mechanical properties similar to those of conventional plastics, such as polypropylene or polyethylene, although it exhibits a high degree of crystallinity, leading to brittleness and low elongation at break [2]. P3HB is a fully biodegradable and biocompatible material, and due to these characteristics, this polymer is a potential candidate as a substitute for petrochemical plastics. However, due to the high production costs of P3HB and other PHAs, the most economically attractive applications are in the biomedical field, as scaffolds for the cellular growth of cartilage and bone; for medical devices, such as sutures, adhesion barriers and valves to guided tissue repair; and for regeneration devices such as cardiovascular patches [1,3–5].

Physicochemical properties and applications of P3HB are affected by its molecular mass, as this feature determines the elastic behavior of the material and its mechanical resistance [6]. For example, fibers of P3HB with a molecular mass of approximately 300 kDa have a tensile strength of 190 MPa and an elongation at break of 5%. In contrast, when the molecular mass is of 5300 kDa, the fibers increase their tensile strength 7-fold (1320 MPa), and the elongation at break is 57% [6].

It has been reported that the molecular mass of P3HB is affected by various culture parameters such as the culture medium composition, pH, temperature and aeration conditions of the bioreactor used for the production of the polymer [7–10]. In addition, the molecular mass of P3HB has been shown to be affected by other factors, such as the relative level of expression of the biosynthetic enzymes, which can be affected by the order of the biosynthetic genes (*phbA, phbB* and *phbC*) within the *phb* operon, and the level of activity of P3HB synthase [11], the type of PHA synthase present [12,13] and mutations on the P3HB synthase enzyme [14].

Among the bacteria that are able to accumulate large amounts of P3HB, *Azotobacter spp* have been reported to synthesize a polymer of ultra-high molecular mass [1,7,10,15]. It is important to note that the process of intracellular accumulation of the polymer is the result of two stages of its metabolism: one is the P3HB synthesis, and the other is the degradation of the polymer. P3HB

* Corresponding author at: Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apdo. Post. 510-3, Cuernavaca, 62210 Morelos, México.
E-mail address: carlosf@ibt.unam.mx (C. Peña).

http://dx.doi.org/10.1016/j.procbio.2016.04.013
1359-5113/© 2016 Elsevier Ltd. All rights reserved.

synthesis starts with the condensation of two molecules of acetyl-CoA, which is catalyzed by the β-ketothiolase (encoded by the *phbA* gene) to generate acetoacetyl-CoA. Subsequently, acetoacetyl-CoA is reduced by an NADPH dependent acetoacetyl-CoA reductase (encoded by the *phbB* gene) to produce β-hydroxybutyryl-CoA. Finally, β-hydroxybutyryl-CoA is polymerized by the P3HB synthase (encoded by the *phbC* gene), releasing CoA and producing the P3HB [16,17]. When the external carbon source is depleted, the bacterium uses the P3HB reserves, mobilizing this polymer for energy production [18,19]. P3HB degradation starts when the polymer chains are converted into β-hydroxybutyrate or P3HB oligomers by P3HB depolymerase (*phbZ* gene) enzyme. Later, β-hydroxybutyrate is oxidized by NAD^+ dependent β-hydroxybutyrate dehydrogenase enzyme, producing acetoacetate. Finally, succinyl-CoA acetoacetate transferase converts acetoacetate to acetoacetyl-CoA [20,21]. Because synthesis and degradation occur simultaneously [22,23], the molecular mass of P3HB accumulated by the bacterium could be affected by the ratio of both stages, but in the case of *A. vinelandii* it is unknown how the stage of synthesis and degradation influence the molecular mass of the polymer.

There are very few reports in which the changes in the molecular mass of P3HB have been studied during the cultures of *A. vinelandii* under fixed dissolved oxygen conditions. Thus far, it is not known whether the level of activity of the enzymes involved in the process of intracellular accumulation influence the molecular mass of polymer produced. In a recent study [10], it was reported that the molecular mass (MM) of P3HB produced by *A. vinelandii* was influenced by the aeration conditions and the strain used. In that study, when *A. vinelandii* OPN mutant and its parental strain OP were cultivated during 60 h under low aeration conditions, they produced polymers with a molecular mass of 2020 kDa and 1650 kDa, respectively. However, when both strains were cultivated under a condition of higher aeration, the molecular mass of P3HB decreased to 1010 kDa and 551 kDa for OPN and OP strains, respectively. It should be noted that study was performed in shaken flasks where culture parameters, such as the pH and the dissolved oxygen tension (DOT), were not controlled. Changes in these parameters during the cultivation might influence the molecular mass of P3HB, as it was reported in cultures of recombinant *Escherichia coli* and *Azotobacter chroococcum* [7,9,24]. Additionally, in that study, they did not determine the activity of the enzymes that participated in the synthesis and degradation of the P3HB.

There are some reports in the literature about the analysis of the enzyme activities of both synthesis and degradation during the process of accumulation of P3HB in other microorganisms [11,25–27]. However, it is not clear how the activities of the enzymes that participate in both the synthesis and degradation influence the molecular mass of P3HB. In *A. vinelandii*, this has not been studied yet. Therefore, the aim of this work was to study the role of the level of enzymatic activities involved in the synthesis and degradation of P3HB on its molecular mass in a bioreactor under controlled dissolved oxygen conditions in cultures of *A. vinelandii* OP.

2. Materials and methods

2.1. Microbial strain, culture medium and inoculum preparation

A. vinelandii OP (ATCC 13705) was used in this study. This strain is unable to produce alginate (an extracellular polysaccharide) because it has an insertion sequence in the *algU* gene, which encodes a transcriptional factor that regulates genes involved in synthesis of alginate and mobility [28]. The strain was cryopreserved at −70 °C in a 40% glycerol solution and maintained by monthly subculture on PYís agar slopes and stored at 4 °C [1]. *A. vinelandii* OP was cultured in PY medium with the following composition in g L^{-1}: sucrose 20.0 (J.T. Baker); yeast extract 3.0 (Difco); peptone 5.0 (Difco). The pH of medium was adjusted to 7.2 with addition of a 2 N solution of NaOH, and the medium was sterilized at 121 °C for 20 min. The inoculum was incubated on a rotatory shaker (New Brunswick Scientific Co., Model G 25, shaking radius = 2.5 cm) at 200 rpm and 29 °C, to an absorbance (measured at 540 nm, after performing a dilution 1:50 before the measurement) between 0.16 and 0.18 (corresponding to a cell dry weight between 0.08 and 0.1 g L^{-1}). A total of 200 mL of the broth culture was centrifuged at 12,860*g* for 10 min at 4 °C. The supernatant was discarded, and the cells were suspended in 200 mL of fresh PY medium and transferred to the bioreactor containing 1.8 L of medium.

2.2. Culture conditions in the bioreactor

The cultures were grown in an Applikon bioreactor containing 2 L of PY medium at 29 ± 0.5 °C, at 500 rpm. The stirred tank bioreactor was equipped with two Rushton turbines ($D_T/T = 1/3$), where D_T is the turbine diameter and T is the bioreactor diameter. The pH was measured with an Ingold probe (Applikon, ADI 1010) and controlled to 7.2 ± 0.1 by an on/off system using a peristaltic pump and by adding 2 N NaOH or HCl solutions. The dissolved oxygen tension (DOT) was measured with an Ingold polarographic probe, controlled through a gas mixture (O_2 and N_2) [29]. *A. vinelandii* is a strict aerobic bacterium that has a critical value of DOT at 4% [30–32]. It is known that when this bacterium is grown under oxygen limited conditions (below the critical oxygen concentration), the P3HB accumulation process is favored. In contrast, under non-oxygen-limited conditions (DOT higher of 4%) cell growth is promoted, followed by the accumulation of the polymer. In this study, conditions of oxygen limitation (DOT = 1 ± 0.2% equivalent to 0.0021 mmol O_2 L^{-1}) and non-oxygen limitation (DOT = 15 ± 0.65% equivalent to 0.0137 mmol O_2 L^{-1}) were evaluated. All experiments were conducted in triplicate, and the results presented are the average of independent runs.

2.3. Oxygen transfer rate and specific oxygen uptake rate determinations

Equations used for determination of oxygen transfer rate (OTR) and specific oxygen uptake rate (q_{O_2}) were as follows:

$$C^* = \frac{\left[\frac{F_{O_2}}{F_T}(P_{atm})\right]}{H} \tag{1}$$

$$OTR = k_L a (C^* - C_L) \tag{2}$$

$$q_{O_2} = \frac{OTR}{X} \tag{3}$$

where C^* (mmol L^{-1}) is the dissolved oxygen concentration at equilibrium, calculated based on the proportion of the flux of oxygen at the inlet (F_{O_2}: L h^{-1}) of the system and the total flux of gas at the inlet (F_T: L h^{-1}), P_{atm} is the pressure in the system (0.825 atm), and H is Henryís constant at 29 °C in water for oxygen. OTR (mmol L^{-1} h^{-1}) is the oxygen transfer rate, $k_L a$ (h^{-1}) is the volumetric oxygen transfer coefficient, C_L (mmol L^{-1}) is the dissolved oxygen concentration, q_{O_2} (mmol g^{-1} h^{-1}) is the specific oxygen uptake rate, and X (g L^{-1}) is the protein concentration.

2.4. Analytical determinations

2.4.1. Biomass, protein, sucrose and poly-3-hydroxybutyrate (P3HB) concentration

Cell dry weight was determined gravimetrically using 6 mL of culture broth, which was centrifuged at 9660*g* during 10 min. The pellet was isolated, mixed in distilled water and filtered through

Download English Version:

https://daneshyari.com/en/article/34140

Download Persian Version:

https://daneshyari.com/article/34140

Daneshyari.com