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In situ observation of lamellar growth in thin films for poly[(*R*)-3-hydroxybutyric acid-*co*-6-hydroxyhexanoic acid] at a high crystallization temperature of 110°C by atomic force microscopy

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Abstract

In situ imaging of the development on lamellar crystals in thin films for poly[(*R*)-3-hydroxybutyric acid-*co*-10 mol%-6-hydroxyhexanoic acid] was carried out at a high crystallization temperature of 110°C by temperature-controlled atomic force microscopy. The microfibril crystals with dimensions of 100–500 nm length, 50–90 nm width and 6–8 nm thickness were observed at the growth front of lamellae. These microfibril crystals grew along the longitudinal direction of lamellae during crystallization. In some cases, newly formed microfibril crystal and branching microfibril crystal were observed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Poly(hydroxyalkanoic acid); Lamellar growth; In situ imaging

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1. Introduction

Poly(hydroxyalkanoic acid)s (PHA), a family of polyesters, are biodegradable and biocompatible thermoplastics and have attracted much attention as environmentally degradable polymeric materials for various applications [1–3]. The biodegradation of PHA takes place on the surface of samples by the function of extracellular enzymes from microorganisms [1,2]. On the basis of the characterization for the enzymatic degradation of films [4] and monolamellar single crystals [5], it has been demonstrated that the rate of biodegradation for PHA materials is strongly dependent both on the chemical structure of monomeric unit and on the solid-state structure of samples. In the aspect of the solid-state structure of PHA materials, the crystallinity and lamellar crystal sizes play a decisive role in the degradation process [6].

In a previous paper [7], we characterized the crystalline morphologies of thin films with 100 nm thickness for poly[(*R*)-3-hydroxybutyric acid] and poly[(*R*)-3-hydroxybutyric acid-*co*-10 mol%-6-hydroxyhexanoic acid] (P[(*R*)-3HB-*co*-6HH]) crystallized at various temperatures from the melt by means of optical, transmission electron and atomic force microscopies (AFM). At high crystallization temperatures such as 110–120°C, only flat-on lamellae were formed in thin film, and microfibril crystals with dimensions of 50–90 nm width and 8–10 nm thickness were observed at the growth front of lamellae. After enzymatic degradation by a PHB depolymerase, the jagged texture along the crystal long axis could be observed at the ends of crystalline lamellae on the surface of thin film. From these observations, we have suggested that the lamellar crystals of poly[(*R*)-3-hydroxybutyric acid] are composed of both tightly molecular packing regions of microfibril crystals and loosely molecular packing boundary regions, and that the formation of different chain packing regions is related to crystal growth manner of PHA materials. This result prompted us to investigate the crystallization mechanism of PHA materials by means of in situ atomic force microscopy observation.

AFM is one of the most useful techniques to characterize the surface morphology and nanostructure of polymer samples [8]. Recently, in situ observations for monitoring the growing crystals have been attempted with the usage of AFM [9–11]. McMaster et al. [9] reported the novel method for real-time imaging of growing spherulites for poly[(*R*)-3-hydroxybutyric acid-*co*-24 mol%-(*R*)-3-hydroxyvaleric acid] (P[(*R*)-3HB-*co*-(*R*)-3HV]) copolymer at room temperature from the melt by using AFM. The same

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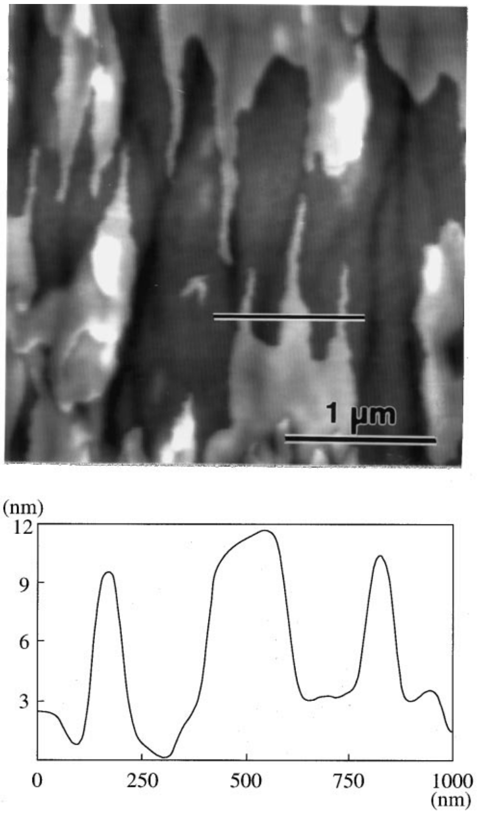

Fig. 1. Typical AFM height image and line profile of P[(*R*)-3HB-*co*-10 mol%-6HH] thin film crystallized at 110°C for several minutes (*t* min) after the temperature was stable at 110°C.

group applied the phase imaging technique of AFM to determine the growth rate of spherulites for P[(*R*)-3HB-*co*-(*R*)-3HV] copolymer [10]. They suggested that the growth of spherulites showed a constant rate as a whole, while that the individual lamellar stacking grew at various rates.

In this communication, we report the first result to observe the development of individual lamellar crystal on the surface of PHA thin film at a high crystallization temperature of 110°C by using AFM.

2. Materials and method

A random copolyester of (*R*)-3-hydroxybutyric acid with 10 mol% of 6-hydroxyhexanoic acid (P[(*R*)-3HB-*co*-6HH], number-average molecular weight = 107,000, polydispersity = 1.7) was prepared by ring-opening copolymerization of β-butyrolactone and ϵ-caprolactone as described previously [12]. Thin layer of P[(*R*)-3HB-*co*-6HH] copolyester with 100 nm thickness was prepared on a surface of glass substrate by spreading the diluted solution of polymer in chloroform as described in a previous paper [7]. Crystal morphology on the surface of P[(*R*)-3HB-*co*-6HH] thin film was observed with an atomic force microscope (Seiko Instruments Inc., SPI3800/SPA300) equipped with heating stage and vacuum chamber. Melting and crystallization of thin film for P[(*R*)-3HB-*co*-6HH] were carried out under reduced pressure (typically 10^{-2}–10^{-3} torr) on the heating stage in the vacuum chamber. The cast thin film was heated at 200°C, and then cooled down to the crystallization temperature of 110°C. AFM imaging was started at several minutes (*t* min) after the sample temperature reached to 110°C. Pyramid-like Si_3N_4 tip mounted on 200 µm long cantilever with a spring constant of 0.38 N/m was used in the contact mode. Simultaneous registration was performed for height and deflection images.

3. Results and discussion

Spherulitic and lamellar morphologies in P[(*R*)-3HB-*co*-6HH] thin films of 100 nm thickness after crystallization at 110°C from the melt at 200°C have been characterized by using an optical microscope and a transmission electron microscope at room temperature. The uniform two-dimensional (2D) spherulites were developed throughout the thin film. On the basis of electron diffraction patterns of P[(*R*)-3HB-*co*-6HH] thin films, it was confirmed that the P[(*R*)-3HB] crystal formed the “flat-on” lamellae in P[(*R*)-3HB-*co*-6HH] thin films and that the lamellar crystals stacked parallel to the based substrate plane. The lamellar crystals have grown along the radius of 2D spherulites, and the long axis of lamellar crystal was their crystallographic *a* axis. Since the *d*-spacing values of P[(*R*)-3HB-*co*-6HH] lamellar crystals calculated from the electron diffractogram were in good agreement with those values of P[(*R*)-3HB] homopolymer, it has been concluded that the randomly distributed 6HH units in P[(*R*)-3HB-*co*-6HH] are excluded from the P[(*R*)-3HB] crystalline phase.

In situ observation of the surface for a P[(*R*)-3HB-*co*-6HH] thin film was performed by AFM equipped with heating stage. All treatments of the P[(*R*)-3HB-*co*-6HH] thin film was carried out on the heating stage under reduced pressure, and the images were recorded isothermally at 110°C after melting at 200°C. It was necessary to keep the cantilever at sample surface for several minutes (*t* min) before scanning so as to stabilize the deformation of cantilever attended by temperature changes. Figs. 1 and 2(a) show the typical AFM height and deflection images of the P[(*R*)-3HB-*co*-6HH] thin film crystallized at 110°C for *t* min, respectively. As shown in images, the 2D spherulites have been formed in thin films during isothermal crystallization at 110°C for *t* min, and flat-on lamellar stacking was observed throughout the scanning area. The lamellar thickness of a P[(*R*)-3HB-*co*-6HH] thin film was measured from the height image of the flat-on lamellae. The lamellar thickness for the thin film crystallized at 110°C ranged from 6 to 8 nm and was almost identical with the values (8.2 ± 0.5 nm) of long period determined by small-angle X-ray scattering for the melt-crystallized thick film. At the

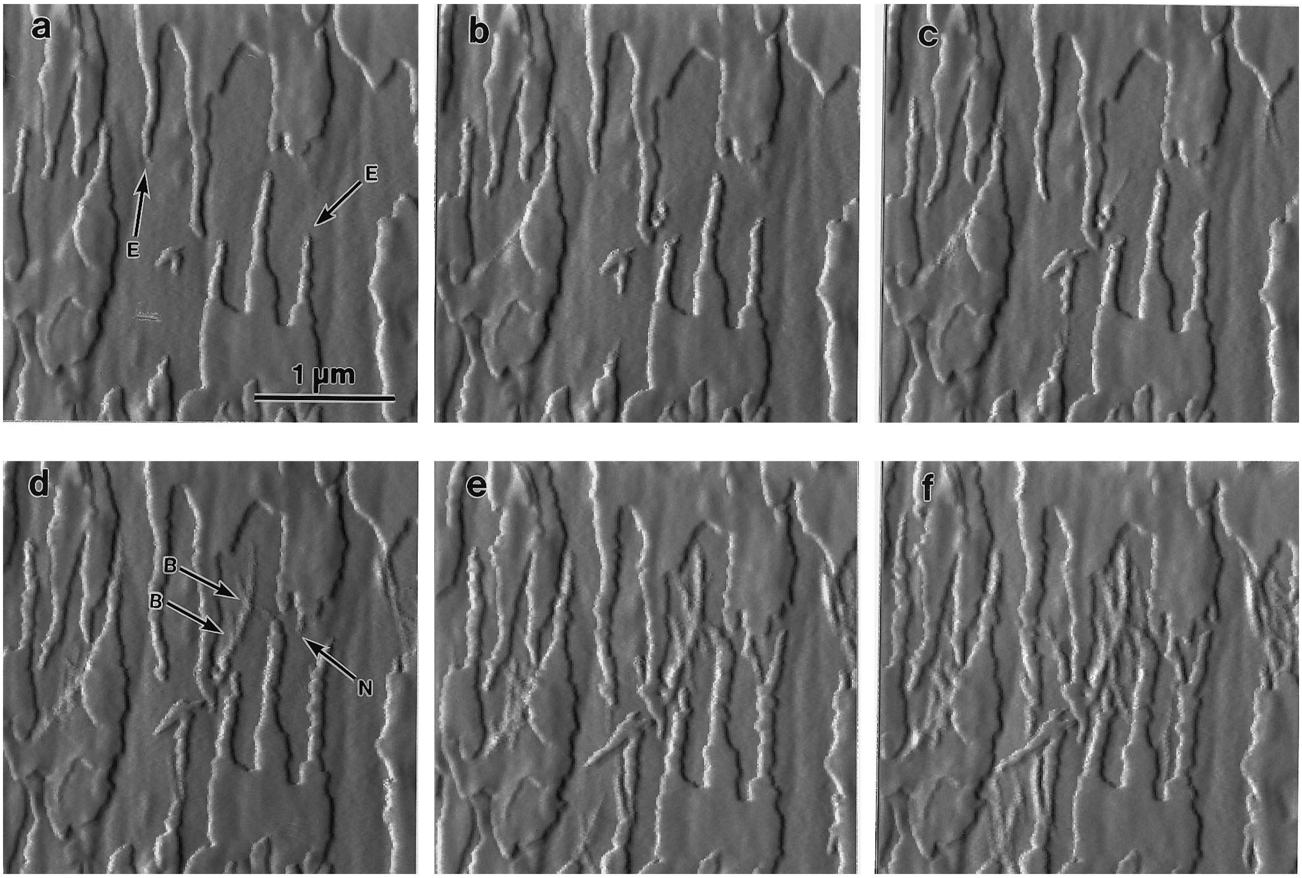

Fig. 2. AFM deflection images of P[(*R*)-3HB-*co*-6HH] thin film, taken during isothermal crystallization at 110°C on the AFM heating stage. In this sequence, the first frame (a) was taken at several minutes (*t* min) after the temperature was stable at 110°C. The following frames (b)–(f) were taken at ($t + 10$), ($t + 20$), ($t + 40$), ($t + 60$) and ($t + 90$) min, respectively. E: typically elongating and expanding microfibril crystals, N: newly formed microfibril crystals, B: branching microfibril crystals.

top surface of thin film, several smaller lamellar crystals were observed. At the growth front of lamellar crystals on the top surface, the microfibril crystals with dimensions of 100–500 nm length, 50–90 nm width and 6–8 nm thickness were detected. The thickness of microfibril crystals was identical with the value of based lamellar crystals.

Fig. 2(b)–(f) shows the AFM deflection images of a P[(*R*)-3HB-*co*-6HH] thin film allowed to stand at 110°C. From the successive images of the P[(*R*)-3HB-*co*-6HH] thin film, the development of lamellae and microfibril crystals formed at the top surface could be observed during isothermal crystallization. The microfibril crystals grew along the longitudinal direction of lamellae until the tip of the microfibril crystals impinged with the other crystals (Fig. 2(b)–(f)). The width of the microfibril crystals also expanded with time. In addition, microfibril crystals growing with branched texture were also detected (Fig. 2(d)). Furthermore, new microfibril crystals appeared both at the growth front of lamellar crystals and at the surface of based lamellar crystals (Fig. 2(b) and (d)).

The initial thickness of new microfibril crystals was around 3–4 nm, and the microfibril crystals were thickened to reach around 6–8 nm with time (Fig. 2(b)–(f)). This result indicates that the lamellar crystals of P[(*R*)-3HB-*co*-6HH] develop with both lateral growth and thickening growth during isothermal crystallization, and may be the first direct visualized data that show the thickening growth of lamellar crystals. The microfibril crystals may be thickening owing to the conformational adjustment of molecules or the sliding of polymer chains. The polymer chains inside the microfibril crystals of P[(*R*)-3HB-*co*-6HH] may be packed tightly during the thickening growth process. The microfibril crystals grew in the lateral direction until the tip of microfibril crystals impinged with the other crystals and then joined. The disordered structures may remain at the joints between the microfibril crystals, while the majority of mismatched structures could be removed from the joint by the conformational adjustment of molecules or the sliding of polymer chains.

The growth rate of individual micrifibril crystals was determined from the successive images. The growth rate of the individual microfibrils for P[(*R*)-3HB-*co*-6HH] ranged at 5–8 nm/s at 110°C. The microfibril growth rate was slower than that of the spherulites (20 nm/s) in P[(*R*)-3HB-*co*-6HH] thick films crystallized at 110°C, reported by Abe et al. [12]. It has been proposed that the crystal growth rate of thin films decreased with a decrease in film thickness due to reduction in the mobility of the molecules [13]. In this study, the based lamellar crystals have been formed at the initial stage for several minutes at 110°C. The microfibril crystals were developing on the surface of based lamellar crystals. The limitation of thickness between the based lamellar crystals and the free surface may cause the depression of growth rate for the microfibril crystals.

Since each stacking lamellar part of the thin films, including the microfibril crystals, yielded a sharp electron diffraction pattern, the crystal structure of the microfibril crystals is identical with that of the flat-on lamellar crystals. In a previous manuscript [7], we have suggested that the lamellar crystals in thin films may be the aggregate of microfibril crystals and proposed the model of lamellar crystal growth for PHA materials. In the model, the microfibril crystals first grow from the secondary nuclei formed at the growth front of the lamellae. After this, the residual amorphous polymer chains become incorporated into the edges of the microfibril crystals or into new secondary nuclei formed at the growth front to fill up the regions between the microfibril crystals. Owing to the depression of the crystal growth rate, the growth of microfibril crystals could be clearly observed at the top surface of the thin film.

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