

Amorphous and semicrystalline phases in ultrathin films of poly(3-hydroxybutirate)

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ABSTRACT

2 METHODS

Poly(3-hydroxybutirate) (PHB) is a semicrystalline biocompatible polymer. In the current work the morphology of ultrathin PHB films was studied by atomic-force microscopy (AFM). The ultrathin films consisted of amorphous drops and semicrystalline lamellae. The crystallization of amorphous drops was visualized at 85°C. At higher temperature (125-150°C) the crystallization was too fast to be followed by AFM however it was possible to observe the fragmentation of the lamellae and the increase in their height. The obtained data can be explained in terms of relaxation of a metastable film. Upon heating the film adopts the energetically preferable morphology by three interconnected processes: crystallization of the amorphous drops, decrease of the PHB-mica contact area and lamellar thickening.

Keywords: poly(3-hydroxybutirate), atomic-force microscopy, spin-coating, ultrathin film, semicrystalline polymer

1 INTRODUCTION

Poly(3-hydroxybutirate) (PHB) is a bacterial polyester, which shows a high potential as a material for biotechnology and medicine, including drug delivery and production of biodegradable implants [1]. Being a semicrystalline polymer, PHB is able to form various supramolecular structures, such as lamellae and spherulites. The semicrystalline structure and morphology of the polymer strongly influences its biodegradation properties [2]. In the current work the morphology of ultrathin PHB films prepared by spin-coating was studied by atomic-force microscopy (AFM). The coexistence of morphologically different components (phases) with different degree of crystallinity was established. Upon heating the film morphology changed, and the changes could be explained by considering the relaxation of the film from the metastable state in which the polymer was trapped by spin-coating.

A PHA producer *A. chroococcum* strain 7B, a non-symbiotic nitrogen-fixing bacterium able to overproduce PHB (to 80% of cell dry weight) was used [3]. The strain was isolated from the wheat rhizosphere (sod-podzolic soil) and maintained on Ashby's medium, containing 0.2 g/l $K_2HPO_4 \cdot 3H_2O$, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.2 g/l NaCl, 0.006 g/l $Na_2MoO_4 \cdot 2H_2O$, 5.0 g/l $CaCO_3$, 20 g/l sucrose, and 20 g/l agar. All experiments were performed under laboratory conditions. For PHB synthesis in cells, the culture was grown in shaker flasks (containing 100 ml of the medium) at 30°C in Burk's medium, containing 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$ – 0.01 g/l, 0.006 g/l $Na_2MoO_4 \cdot 2H_2O$, 0.5 g/l sodium citrate, 0.1 g/l $CaCl_2$, 1.05 g/l $K_2HPO_4 \cdot 3H_2O$, 0.2 g/l KH_2PO_4 , and 17 g/l (50 mM) sucrose as the primary carbon source. To control M_w of PHB sodium acetate (SA) at a concentration of 2.5 g/l (30 mM) was added to medium. The experiment was performed for 72 h. Optical density was controlled by nephelometry. To control strain growth and polymer accumulation in cells a Biomed 1 (Biomed, Russia) light microscope was used [3]. The polymer isolation and purification from *A. chroococcum* comprised the following stages: (1) polymer extraction with chloroform in a shaker for 12 h at 37°C; (2) separation of polymer solutions from cell debris by filtration; (3) polymer precipitation from chloroform solution with isopropanol; (4) subsequent repeated cycles of dissolution in chloroform and precipitation with isopropanol for 4-5 times to remove any additives and contaminants, and (5) drying at 60°C [3]. Molecular weights of PHB were determined by viscosimetry: the viscosity of the PHB solution in chloroform was measured at 30 °C on an RT RheoTec viscometer (RheoTec, Germany); the molecular weight was calculated using the MarkKuhnHouwink equation according to [3].

Ultrathin PHB ($M_w=8.3 \times 10^5$) films were prepared by spin-coating from solution in dichloromethane onto mica and examined by AFM. The AFM imaging was performed with Solver PRO microscope (NT-MDT, Russia) equipped with a heating stage (operating temperature up to 120°C).

To heat the samples above 120°C, the heater of MR Hei-Standard stirrer (Heidolph) was used.

Silicon cantilevers NSG11 (NT-MDT, Russia) with a typical spring constant of 5.1 N/m were used for AFM imaging. The images were recorded in semi-contact mode, a scanning frequency was 1-3 Hz, scanning areas from 3×3 to $20 \times 20 \mu\text{m}^2$. Topography and phase images (512x512 pixels) were captured during each scan. Image processing was carried out using Image Analysis (NT-MDT, Russia) and FemtoScan Online (Advanced technologies center) software.

3 RESULTS

A typical image of heterogeneous film consisting of two distinct components is shown in figure 1. The drops which have roughly spherical shape, were interpreted as the amorphous component, while the continuous seaweed structure was interpreted as the semicrystalline component. The difference between the amorphous and semicrystalline PHB is clear when imaging in phase contrast mode (figure 1 B). The observed phase contrast presumably originates from the difference in mechanical properties (adhesion and rigidity) of the components. The thickness of the lamella was found to be 4-5 nm in agreement with early data [4].

In agreement with [5], we observed the disappearance of the amorphous drops when the film thickness increased. The semicrystalline phase adopted one of the well-known morphologies typical to diffusion-limited crystallization (seaweed morphology, two-dimensional spherulites etc).

The ultrathin films prepared by spin-coating are metastable (the lamellar thickness is low, a large fraction of polymer is trapped in amorphous state) and heating facilitates relaxation. In the current case relaxation included several processes: crystallization of the amorphous drops, decrease of the polymer-substrate contact area, fragmentation of the lamellae and increase of their thickness. Temperature is the main parameter that controls the rate of these processes (the melting temperature of PHB is $T_m \sim 176^\circ\text{C}$), adjusting temperature made it possible to observe them by AFM.

Figure 2 shows several height images tracking the growth of a PHB crystal at $T=85^\circ\text{C}$. The first image obtained at room temperature shows a crystal surrounded by shapeless amorphous drops formed by spin-coating. When the sample was heated to 85°C , the amorphous drops became almost invisible, which could be explained by their softening.

During the crystallization process the polymer molecules attached to the crystal edge keeping the crystal height roughly constant (4-5 nm). The speed of the crystal edge was found to be 0.18-0.8 nm/second.

When a PHB film was heated to a higher temperature (above 100°C) the crystallization became too fast to be observed by AFM. However it was possible to observe *recrystallization*. To do so the samples were heated

to 125-150°C, kept for 5 minutes and cooled down to room temperature. The very same region was examined before and after heating. A pair of typical images is shown in figure 3. It is clearly seen that each seaweed-shaped lamella split into separate nanocrystals. This process was accompanied by an increase in sample height, the mean increase of height was $3,1 \pm 0,6$ nm when heating to 125°C and $5,4 \pm 0,7$ nm when heating to 150°C .

Some thin edge-on lamellae are shown in figure 3 A. They had low height (0,5-0,7 nm) and were observed rarely and disappeared after heating.

All the alterations of film morphology observed during recrystallization can be shortly explained as follows. PHB film on mica surface exhibits dewetting and tends to decrease the polymer-substrate contact area. This explanation is in agreement with dewetting of polyesters from silicon substrate [6]. At the same, time lamellar thickening takes place and causes additional increase in film height.

In the current work the coexistence of two phases (amorphous and semicrystalline) in ultrathin films of PHB on mica was established. These phases are morphologically different but show similar tendency to dewetting upon heating. The polymer film adopts the energetically preferable morphology by three interconnected processes: crystallization of the amorphous drops, decrease of the PHB-mica contact area and lamellar thickening.

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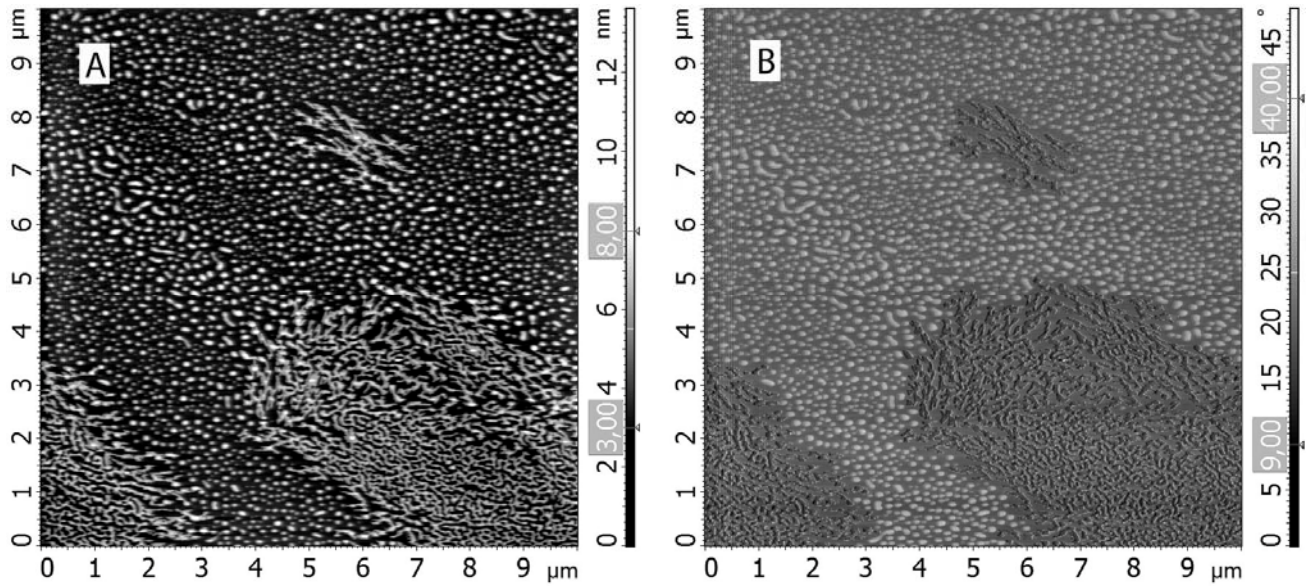


Figure 1: The amorphous and semicrystalline phases in ultrathin PHB film on mica. A – topographical image, B – phase contrast image.

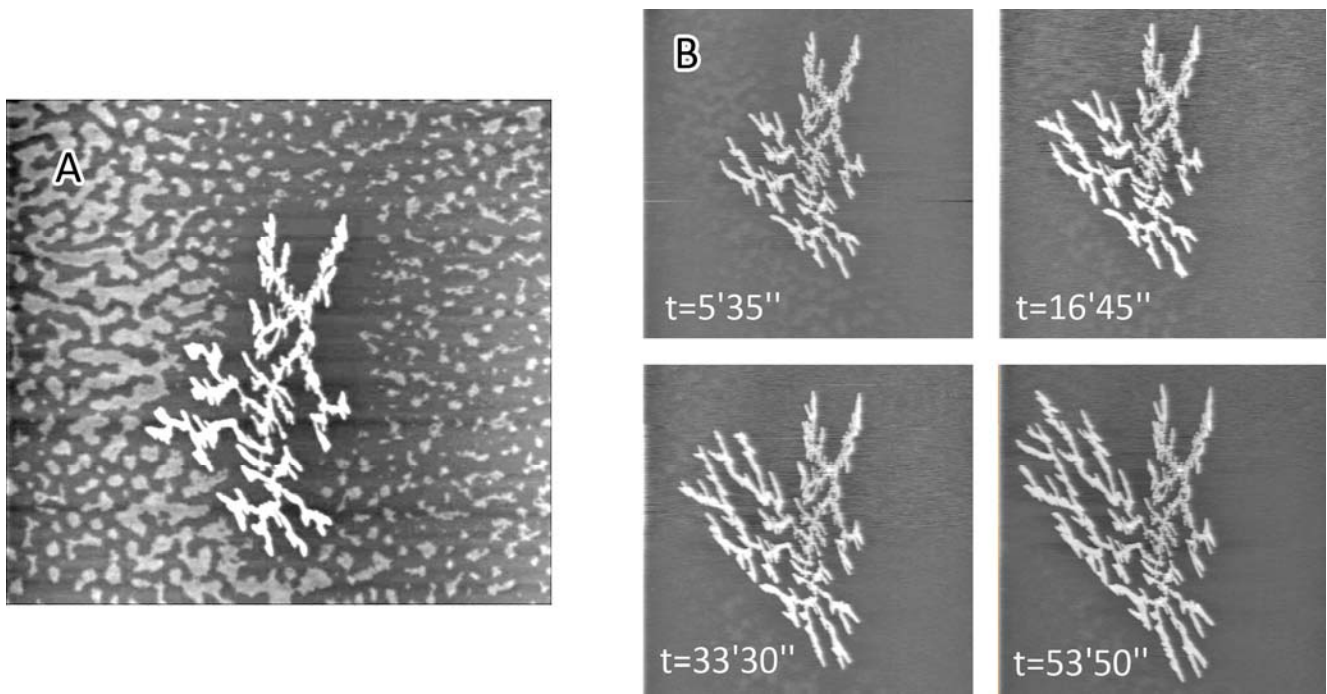


Figure 2: A series of consecutive images showing the growth of a PHB crystal. Scan size $5 \times 5 \text{ μm}^2$ for all the images, Z scale 5 nm. A – the initial state of the film after spin-coating. The amorphous part surrounding the crystal is clearly seen. B – PHB crystallization at 85°C .

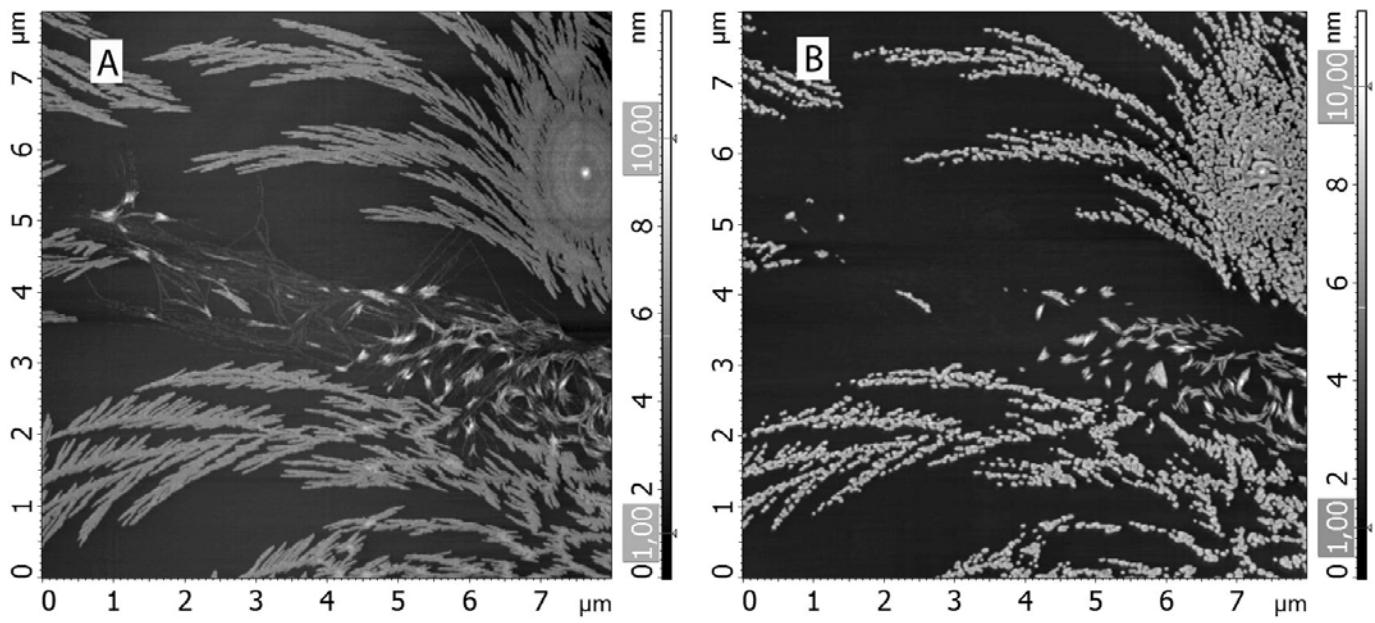


Figure 3. Film rearrangement on heating. Both images were recorded at room temperature before (A) and after (B) heating the sample to 125°C for $t=2$ min.