Phase Structure and Enzymatic Degradation of Poly(L-lactide)/ Atactic Poly(3-hydroxybutyrate) Blends: An Atomic Force Microscopy Study

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Phase structures and enzymatic degradation of poly(L-lactide) (PLLA)/atactic poly(3-hydroxybutyrate) (ata-PHB) blends with different compositions were characterized by using atomic force microscopy (AFM). Differential scanning calorimetry (DSC) thermograms of PLLA/ata-PHB blends with different compositions showed two glass transition temperatures, indicating that the PLLA/ata-PHB blends are immiscible in the melt. Surface morphologies of the thin films for PLLA/ata-PHB blends were determined by AFM. Phase separated morphology was recognized from the AFM topography and phase images. The domain size of the components was dependent on the blend ratio. Enzymatic degradation of the PLLA/ata-PHB blends was performed by using both PHB depolymerase and proteinase K. Either PLLA or ata-PHB domains were eroded depending on the kinds of enzyme. Surface morphologies after enzymatic degradation have revealed the phase structure along the depth direction. Enzymatic adsorption of PHB depolymerase was examined on the surface of PLLA/ata-PHB blends. The enzyme molecules were found on both domains of the binary blends. The larger number of enzyme molecules was found on the PLLA domains relative to those on the ata-PHB domains, suggesting the higher affinity of the enzyme against PLLA domain.

Introduction

Poly(lactide) (PLA) has attracted much attention due to its degradability in the environment as well as biocompatibility for medical applications.[1−8] PLAs are synthesized from the lactide monomer, which has three enantiomers, the L, D, and meso forms. Therefore, PLAs of L and D homopolymers as well as stereocopolymers can be obtained via polymerization of L, D, and meso lactides. The optical purity in the stereocopolymer affects the crystallinity of the PLAs. For example, the melting temperature and crystallinity are reduced with an increase in the D units in the L rich PLA sequence. Typically, PLAs of an optical purity below 70% are reported to be uncrystallizable.[9]

Hydrolysis of poly(L-lactide) (PLLA) materials is accelerated in the presence of proteinase K from the mold *Tritirachium album*.[10] MacCarty's group[11−14] has reported that proteinase K preferentially hydrolyzes the amorphous PLLA, and that the rate of enzymatic hydrolysis decreases with an increase in the crystallinity of PLLA. In our previous paper,[15] we studied enzymatic degradation of a PLLA amorphous film by using the quartz crystal microbalance (QCM) and atomic force microscopy (AFM) to understand the characteristic interactions between proteinase K and PLLA materials. It has been found that proteinase K molecules irreversibly adsorb on the PLLA surface for the hydrolysis reaction, and that adsorbed enzymes move around on the PLLA surface to hydrolyze the polymer chains around them.

The biopolyester poly[(*R*)-3-hydroxybutyrate] (b-PHB) is a biodegradable and biocompatible thermoplastic, which is produced from renewable carbon sources by a number of bacteria.[16−20] The b-PHB can be degraded by extracellular PHB depolymerase.[21−26] It has been reported that PHB depolymerase is organized with three characteristic domains. Substrate binding domain plays a role in adsorbing to the material surface. The function of catalytic domain is hydrolysis of the PHB chains. Both domains are connected with linker domain. The two-step enzymatic degradation model has been proposed, namely, adsorption onto polyester surface by the function of binding domain and hydrolysis reaction by the function of catalytic domain. Recently, our AFM experiment revealed that the binding domain has the ability to disturb the polyester surface morphology, suggesting that PHB depolymerase firmly binds to the polyester surface through specific chemical interaction between the binding domain and polyester surface.[27,28]

In contrast to crystalline b-PHB, atactic poly(3-hydroxybutyrate) (ata-PHB) is chemically synthesized by the polymerization of racemic β-butyrolactone, and it is a fully amorphous polymer. Amorphous ata-PHB is hardly hydrolyzed by PHB depolymerase, while the ata-PHB blended with a crystalline or glassy polymer component can be hydrolyzed by the enzyme because stable enzyme binding sites are provided by the second

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Table 1. Molecular Weights of PLA and ata-PHB Samples Used in This Study

samples	$M_n \times 10^{-3}$	M_w/M_n
PLLA-400k	456	1.5
PLLA-100k	107	1.5
PDLLA	103	1.5
ata-PHB	67	1.4

component in the blend.[29−31] To our knowledge, the surface morphologies and structures have not been studied in the ata-PHB based blends.

To generate biodegradable materials with a wide range of physical properties, several approaches have been used, such as copolymerization and blending. In terms of a polymer blend, miscibility of blends is one of the most important factors affecting the final polymer properties. In particular, surface structure and morphology of the biodegradable polymer blends have a great impact on the enzymatic degradation behavior by enzymes. AFM is one of the techniques that provides information about surface morphologies and properties with nanometer resolution. Therefore, a number of papers are published in the field of polymer blends by using AFM.[32−35] In addition to the topographic imaging, the phase imaging mode takes an active part in the mapping of surface heterogeneity of the blends because the phase response of the cantilever is sensitive to the surface characteristics, such as adhesiveness, viscoelasticity, friction, and so on.

In this article, we study the phase structures and enzymatic degradation of PLLA/ata-PHB blends with different compositions by using AFM in order to get insight into the enzymatic degradation behavior of PLLA and ata-PHB domains. Enzymatic degradations of the PLLA/ata-PHB blends are performed in the presence of either proteinase K and PHB depolymerase. Proteinase K should hydrolyze the PLLA domain, while PHB depolymerase should erode the ata-PHB domain. In addition to the AFM analysis, phase structures of PLLA/ata-PHB blends would be confirmed by such enzymatic degradation treatments. Since the PHB depolymerase has been reported to adsorb on the stable binding site, adsorption of the enzyme is studied to further clarify the enzymatic degradation manners of PLLA/ata-PHB blends.

Experimental Section

Material. Two kinds of PLLAs with different molecular weights were purchased from Polysciences Inc. (Warrington, PA), and used without further purification. PDLLA (Lot No. #9800: the optical purity of the L-lactyl unit is 50−60%) was supplied from Shimadzu Inc. ata-PHB was chemically synthesized as reported previously,[29] and the ^{13}C nuclear magnetic resonance spectroscopy revealed that the compound was atactic. The number-average molecular weight (M_n) and polydispersity (M_w/M_n) of the PLLAs, PDLLA, and ata-PHB were evaluated by gel permeation chromatography (GPC) at 40 °C, using a Shimadzu 10A GPC system and a 6A refractive index detector with two joint columns of Shodex K-80M and K-802. Chloroform was used as an eluent at a flow rate of 0.8 mL/min. A molecular weight calibration curve was obtained with polystyrene standards of low polydispersity. The molecular weight data are shown in Table 1.

Preparation of PLLA/ata-PHB Blend Thin Films. The PLA/ata-PHB thin film of ca. 200 nm thickness was prepared on cover glass by a spin-cast method. The PLA and ata-PHB components were dissolved in chloroform to a final concentration of 2.0%(w/v), respectively. The solutions were mixed together with different ratio to make a PLA/ata-PHB blend solution. The blend chloroform solution was spin-cast on the glass substrates at a rotation speed of 4000 rpm. The thickness of the thin film was ca. 200 nm measured by AFM. The cast thin films were treated at 220 °C for 30 s and, then, rapidly quenched to 0 °C on an ice block, resulting in the formation of completely amorphous PLLA/ata-PHB blend thin films. [15, 27]

Table 2. Thermal Properties of PLLA-400K/ata-PHB Blends Determined from DSC Thermograms (Second Scan)

composition (w/w)	T_g (°C)	T_m[a] (°C)	ΔH_m[a] (J/g)
100/0	59	179	27
95/5	5, 59	179	34
90/10	0, 57	178	29
85/15	1, 58	178	33
75/25	−1, 57	178	28
50/50	0, 54	178	19
0/100	−1		

[a] T_m and ΔH_m values are derived from the crystals formed during heating process, i.e., cold crystallization.

Thermal Analysis of PLA/ata-PHB Blend Thin Films. The glass-transition temperature (T_g) and melting temperature (T_m) of the blend samples were evaluated by DSC (Perkin-Elmer, Pyris 1). The solvent cast films were encapsulated in the aluminum pans and heated from 25 to 200 °C at a heating rate of 20 °C/min (first scan). Then, the melt sample was kept at 200 °C for 1 min followed by rapid quenching to −50 °C. The samples were heated from −50 to 200 °C at a heating rate of 20 °C/min (second scan). The T_g was taken at the midpoint of the heating capacity change.

Enzymatic Degradation Experiment. Extracellular PHB depolymerase from *R. pickettii* T1 was purified to electrophoretic homogeneity according to the methods by Tanio et al.[21] Proteinase K was purchased from Roche Diagnosis (Mannheim, Germany) and used as received.

Enzymatic degradation of PLLA/ata-PHB blend thin films was performed by immersing the thin films into enzyme solutions of either PHB depolymerase at 37 °C for 24 h or proteinase K at 25 °C for 60 min. For the PHB depolymerase, 1 μg/mL enzyme solution was prepared in 50 mM phosphate buffered solution (pH 7.4), while for proteinase K, the enzyme was dissolved in 50 mM Tris-HCl buffer (pH 8.5) to a final concentration of 200 μg/mL. After enzymatic degradation, the thin films were washed with Milli Q water to be observed by AFM.

To measure the erosion depth of the thin films by proteinase K, enzyme solution was dropped on the PLLA/ata-PHB blend thin film. After the sample was washed with Milli Q water, the AFM study was performed at the interface region formed by the enzymatic degradation.

Adsorption of PHB depolymerase molecules on the blend thin films was carried out. The blend thin films were immersed in the PHB depolymerase solution of 1 μg/mL. The blend thin film was treated with the enzyme solution at 25 °C for 5 min, and then, the thin film was washed with Milli Q water.

AFM Observation. Surface morphologies of PLLA/ata-PHB thin film were observed by dynamic force mode (tapping mode) AFM (SII Nanotechnology Inc., Chiba, Japan, SPI3800/SPA400) in air (25 °C). A 400 μm long silicon cantilever with spring constants of 2.2 N/m was used for the AFM observation. The scan rate ranged from 0.5 to 1.0 Hz. Height and phase images were simultaneously obtained. For the force-displacement curve measurement, a triangular cantilever mounting Si_3N_4 tip with spring constant of 0.02 N/m was applied.

Results and Discussion

Phase Structure of PLA/ata-PHB Blends. The glass transition temperature (T_g) and melting temperature (T_m) of PLLA-400K/ata-PHB blends were determined by DSC, and the data are summarized in Table 2. The blends exhibited two T_g values, suggesting a biphasic separation in the melt.[36] A similar result was obtained in the PLLA-100K/ata-PHB blends. In the

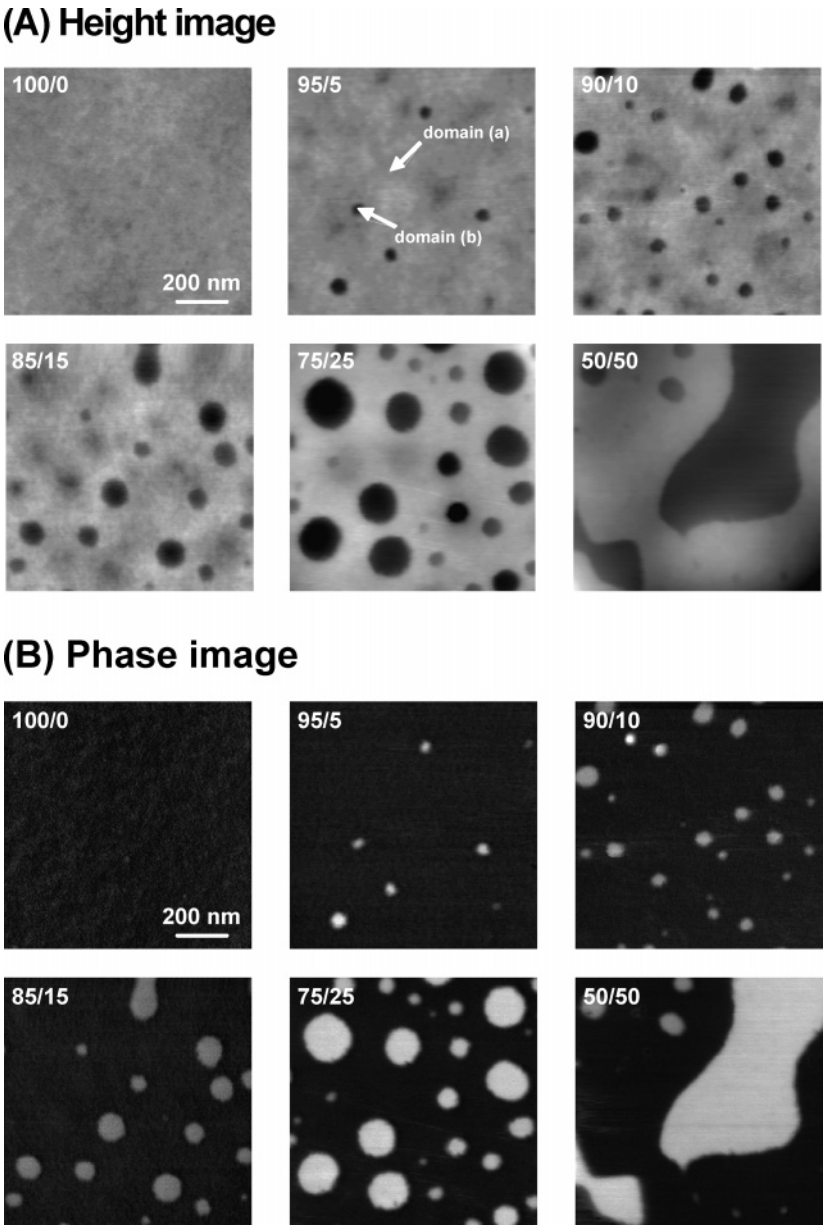

Figure 1. AFM height (A) and phase (B) images of PLLA-400K/ata-PHB blends with different compositions. The blend composition of PLLA and ata-PHB is listed at the upper left corner in each AFM image (PLLA/ata-PHB order). In frames A, the height scale was set to 100/0, 95/5, 90/10, 85/15 = 7 nm; 75/25 = 15 nm; 50/50 = 30 nm. In frames B, each image has its own phase scale to reveal features.

following experiment, PLLA-400K was blended with ata-PHB except for specifying the component.

Surface morphologies of PLLA/ata-PHB blends with different compositions were observed by AFM. Figure 1 shows the AFM height and phase images of PLLA/ata-PHB blends after melting at 220 °C. The phase separated morphology was recognized in the blend thin films. In the AFM height image of Figure 1A, dark and bright contrasts are corresponding to the low and high regions, respectively. The dark area named domain b was increased with an increase in the ata-PHB ratio in the blends, indicating that dark area (domain b) in the AFM height image corresponds to ata-PHB domains, and that the bright area of domain a corresponds to PLLA domains. However, the surface area fraction of pit domains was not equal to the ata-PHB compositions in the blends (Table 3). This result suggests that ata-PHB domain is distributed in the blend thin film along the depth direction.

Typical size and depth of the pit domain b was measured by AFM, and the results are summarized in Table 3. An ata-PHB domain below 30 nm in diameter was not found at the surface of the blend thin films. The diameter and depth of the pit domain increased with increasing ata-PHB composition in the blends. Such a depth profile may be related to the higher molecular mobility of the ata-PHB than PLLA at the AFM observation temperature of 25 °C. Elastic properties of ata-PHB induced the depression of the surface level from the glassy PLLA during the cooling process from melted temperature of 220 to 0 °C.

AFM phase imaging has been used for mapping the variations in composition, adhesion, friction, viscoelasticity, and so on. Figure 1B shows the AFM phase image of PLLA/ata-PHB blends. Different phase contrasts were found between the PLLA and ata-PHB domains. From the viewpoint of domain size, bright and dark regions are ata-PHB and PLLA domains, respectively. Phase image contrast is dependent on the chemical and mechanical properties of the samples.[37] Taking into account T_g for PLLA and ata-PHB, PLLA domains are harder than ata-PHB ones at 25 °C. Therefore, the phase response of PLLA should be brighter than that of ata-PHB. However, the experi-

Table 3. Surface Features of PLLA-400K/Ata-PHB Blends

PLLA/ataPHB composition (w/ w)	surface area fraction of ata-PHB domain (%)	typical diameter of pit (ata-PHB) domain (nm)	depth of pit (ata-PHB) domain from the PLLA surface (nm) before enzymatic degradation (original)	depth of pit (ata-PHB) domain from the PLLA surface (nm) after exposure to PHB depolymerase for 24 h
100/0	0	0	0	0
95/5	2.5 ± 0.6	75 ± 17	3.5 ± 1.1	19 ± 12
90/10	6.7 ± 0.6	76 ± 22	3.5 ± 1.1	49 ± 10
85/15	9.8 ± 0.5	94 ± 26	4.8 ± 2.1	102 ± 15
75/25	22.0 ± 3.6	127 ± 46	7.3 ± 3.0	152 ± 50
50/50	34.7 ± 8.7	*a*	11.8 ± 4.5	183 ± 92

[a] Since the pit domains did not show round but random shape, it was difficult to measure the diameter of pit domain.

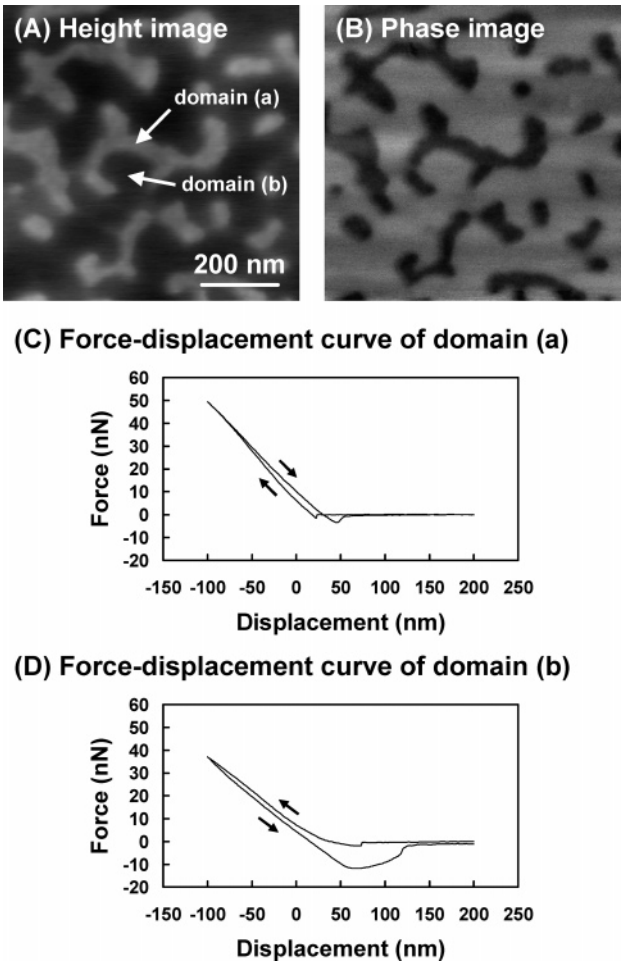

Figure 2. AFM height (A) and phase (B) images of PDLLA/ata-PHB blend (50/50). Frames C and D show the typical force-displacement curves for domains a and b, respectively. The vertical scale in the height image is 10 nm (dark to bright).

mental result in Figure 1B was opposite. Such a reversible phase response in the AFM phase image may be understood as described below.

In the case of PDLLA/ata-PHB blend (50:50 ratio), a phase separated morphology was also observed as shown in Figure 2A,B, suggesting that this blend is also immiscible. This result was supported by the DSC result, which showed two T_g values derived from PDLLA and ata-PHB. Domain a in Figure 2A was decreased with increasing ata-PHB composition in the blend, suggesting that the bright region in height image (domain a) is a PDLLA domain, while domain b is assigned to ata-PHB. In this case, the AFM phase image of Figure 2B showed the existence of two regions with different surface properties. To determine the origin of phase contrast, force-displacement curves were obtained in both domains a and b.[32,33] Figure 2C,D shows the force curves corresponding to domains a and b, respectively. Notable differences in shape and slope of loading and unloading curves were recognized. The slope of the left-hand part of the curve during approach is related to the stiffness of the sample surface, while the downward projection during retraction is attributed to the pull-off force (adhesion interaction), which is the force difference between the noncontact state and the minimum in the force. As shown in Figure 2C,D, steeper slope and lower pull-off force were observed in domain a as compared to domain b, indicating that domain a shows stiffer and lower adhesion properties than domain b. As stated in the former section, the darker phase response has been reported to come from softer material than other brighter domains.[37] However, phase contrast sometimes reversed depending on the adhesion force on the sample surface and the magnitude of cantilever oscillation. For example, the height and phase contrast of hard/soft polymer blend were reversed as the tapping force level was changed from light to hard state.[32,33,39,40] Therefore, on the basis of the force curve data in Figure 2C,D, it can be concluded that domain a is PDLLA, while domain b is ata-PHB.

Enzymatic Degradation of PLLA/ata-PHB Blends. To further confirm the phase structure of the PLLA/ata-PHB blends, enzymatic degradation of the thin film was performed in the presence of either PHB depolymerase or proteinase K. Thin films of PLLA/ata-PHB blends were immersed into the 50 mM phosphate buffered solution containing PHB depolymerase at 37 °C for 24 h. Figure 3 shows the AFM height images of PLLA/ata-PHB blends with different compositions after enzymatic treatment by PHB depolymerase for 24 h. Since the PHB depolymerase has no ability to hydrolyze the PLLA chains, only adsorption of PHB depolymerases should be observed on the PLLA surface.[38] In contrast, ata-PHB chains in the PLLA or poly(methyl methacrylate) (PMMA) blends are hydrolyzed by the enzyme.[30,31]

In Figure 3, spherical particles of PHB depolymerase molecules were observed on the PLLA domain, similar to the pure PLLA surface.[27,28] The depth of the ata-PHB domain was measured after the hydrolysis reaction, and the data are listed in Table 3. The depth of the ata-PHB domain was increased after enzymatic degradation, suggesting that ata-PHB domain is eroded by the function of PHB depolymerase. It is of importance to note that the depth of ata-PHB domain except for the 50/50 blend is not comparable to the thin film thickness. This result indicates that the ata-PHB domain is not penetrated through the blend thin film with a lower ata-PHB content, and that the ata-PHB component is also phase separated and dispersed in the depth direction of the thin film. In the case of a 50/50 blend thin film, the erosion depth was almost identical with the initial film thickness before enzymatic degradation, suggesting that the substrate under the ata-PHB domain is

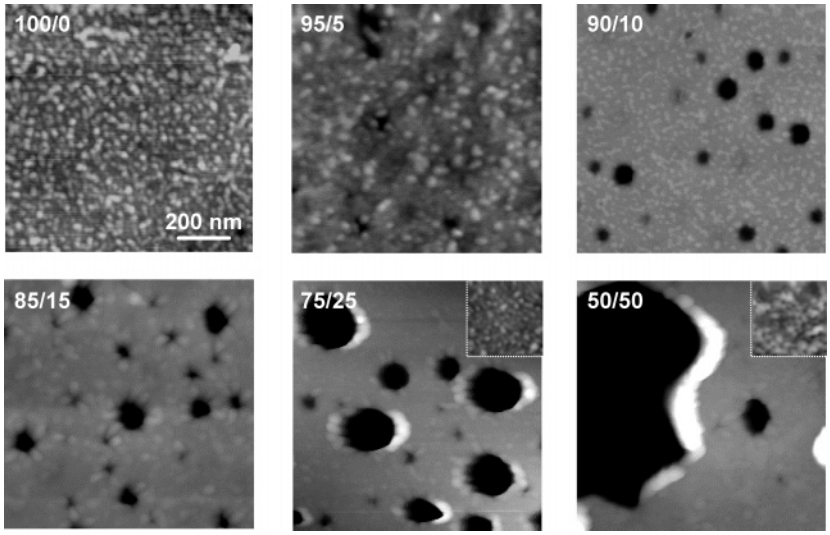

Figure 3. AFM height images of PLLA-400K/ata-PHB blends after enzymatic degradation by PHB depolymerase for 24 h. The blend composition of the blend is listed at the upper left corner of the AFM images in the PLLA/ata-PHB order. To reveal features, height scales have been optimized for each image: 100/0, 95/5 = 10 nm; 90/10, 85/15 = 50 nm; 75/25, 50/50 = 100 nm. Inset for 75/25 and 50/50 shows the different height contrast image at the right corner region (vertical scale is 10 nm), and enzyme molecules adsorbed on PLLA domain was visible.

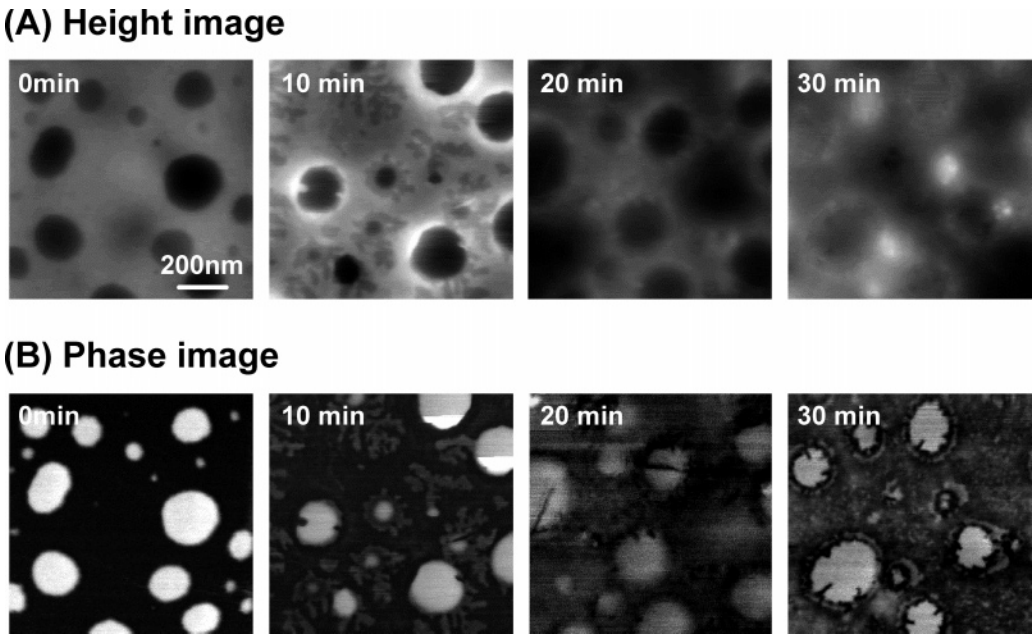

Figure 4. AFM height (A) and phase (B) images of PLLA-400K/ata-PHB blends (75/25) before and after enzymatic degradation by proteinase K for 10−30 min. Each blend samples were treated with proteinase K for different periods, and AFM observation was performed in air at 25 °C. The vertical scale in the height images is 30 nm (dark to bright).

exposed to the surface. Therefore, tuning the appropriate blend ratio of PLLA/ata-PHB and enzymatic degradation by PHB depolymerase provides the porous PLLA material. As for the perspective, exposed substrate surface can be modified by some other materials such as self-assembled layers, resulting in the formation of a functional surface with controlled biocompatibility, which may be used for medical application related to cell attachment.

In the case of proteinase K, the blend thin films were immersed in the 0.1 mM Tris-HCl buffer solution containing the enzyme at 25 °C. Figure 4 shows the AFM height and phase images of PLLA/ata-PHB blend (75/25) after enzymatic degradation by proteinase K for every 10 min. As shown in the AFM height image of Figure 4A, the height difference between the PLLA and ata-PHB domains decreased during the enzymatic degradation of PLLA component and the interface of the domains became unclear. AFM phase images in Figure 4B have demonstrated that the surface of the PLLA domain was eroded heterogeneously, and that both domains were recognized from the phase response even after enzymatic degradation for 30 min. Indeed, the PLLA domain showed less phase contrast against ata-PHB domain. Our previous AFM experiment has revealed that enzymatic degradation of a PLLA thin film with 100 nm thickness by proteinase K was completed within 15 min.[15] However, in the present experiment of the PLLA/ata-PHB blend thin film, PLLA domain remained even after degradation for 30 min, and drastic morphological change was not observed.

To determine the enzymatic degradation rate for the PLLA domain in the blends, stepwise enzymatic degradation experiment was performed. The proteinase K solution was dropped on the blend thin films. After the desired time of incubation, the enzyme solution was washed away by Milli Q water to be observed by AFM. Figure 5A shows the typical AFM height image of PLLA/ata-PHB (75/25) blend thin film after enzymatic

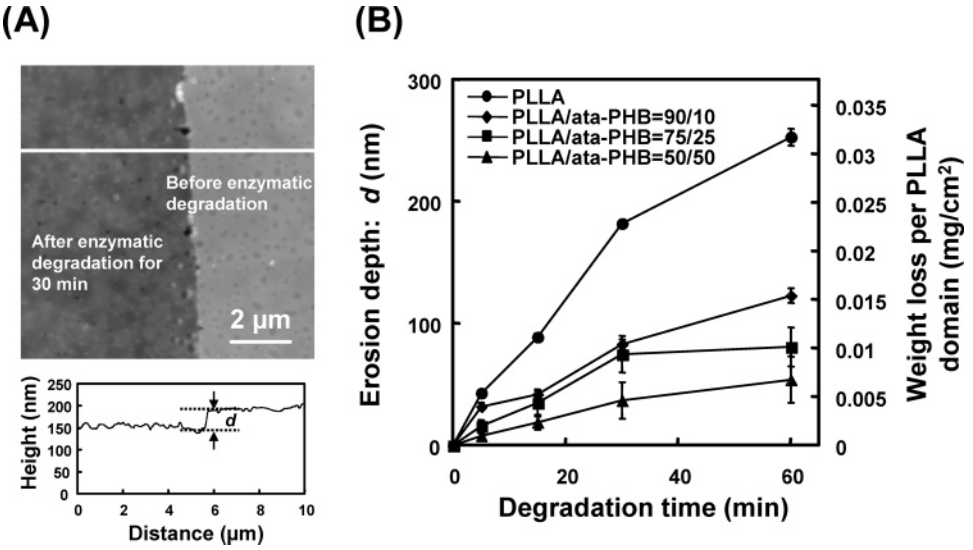

Figure 5. Typical AFM height image (A) of PLLA-400K/ata-PHB blends (75/25) after stepwise enzymatic degradation by proteinase K. Right and left sides in the AFM image correspond to the surface topography before and after enzymatic degradation for 30 min. The graph under the AFM image is cross-sectional at the white line in the image. Both erosion depth (*d*) of PLLA domain and weight loss/PLLA domain calculated from eq 1 were plotted as a function of degradation time for the blend thin films with different compositions, and the result is shown in frame B.[41]

degradation by proteinase K for 30 min. The erosion depth (*d*) was measured at the interface between the degraded and nondegraded regions because the weight loss per unit area can be expressed as follows:

$$\text{weight loss/unit area} = \text{density of polymer}^{41} \times \text{volume reduction due to erosion/unit area} = \text{density of polymer} \times \text{erosion depth} \quad (1)$$

Figure 5B shows the erosion depth (*d*) measured from the AFM height image after enzymatic degradation. The erosion depth of the blends was lower than that of pure PLLA thin film.

In our previous report,[15] enzymatic degradation of PLLA thin film by proteinase K was studied by using QCM and AFM. QCM experiment and stepwise enzymatic degradation test by AFM have revealed the homogeneous degradation of the thin film by proteinase K. However, in the present study, Figure 4 showed heterogeneous degradation manner, and erosion depth was limited below 50−100 nm for blend thin films. The possible explanation is as follows. At 25 °C, segmental motion of PLLA (T_g = 60 °C) is frozen, whereas that of ata-PHB (T_g = 4 °C) is active, suggesting that the ata-PHB domain has higher molecular mobility at 25 °C than PLLA. Such a mobile ata-PHB component leaked out to PLLA surface as the erosion of PLLA domain proceeded, then the ata-PHB retarded both the proteinase K adsorption and following hydrolysis reaction on the PLLA domain. Therefore, the erosion depth of the PLLA domain in the blends may be prohibited to 50−100 nm.

Adsorption of PHB Depolymerase on the Blend Thin Film. AFM analysis in Figure 3 demonstrated the hydrolysis of the ata-PHB domain in the PLLA blends. To understand the enzymatic degradation of the ata-PHB component, a study on the adsorption of PHB depolymerase is one of the effective strategies. Our previous QCM analysis has revealed that PHB depolymerase molecules irreversibly adsorb on polyester surface even after changing the enzyme solution to buffer without enzyme. Figure 6 shows the PHB depolymerase molecules adsorbed on the PLLA and PLLA/ata-PHB (50/50) blend films, which were treated at 25 °C for 5 min in the enzyme solution followed by washing with Milli Q water. In Figure 6A, enzyme molecules of ca. 3 nm in height were observed throughout the pure PLLA thin film. The AFM phase image in Figure 6A

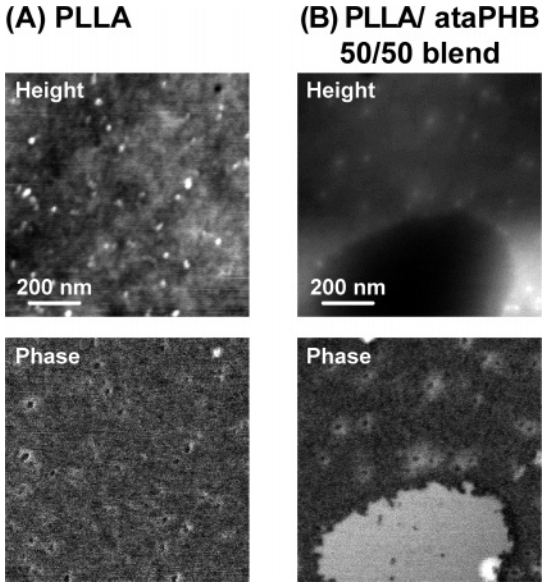

Figure 6. AFM height and phase images of PLLA-400K (A) and PLLA-400K/ata-PHB blends (B) after enzymatic treatment by PHB depolymerase for 5 min. Upper side is AFM height image, while the down side is AFM phase image. The color contrast for height images of frames A and B represents a total range of 5 and 30 nm, respectively.

indicates that the surface properties around the adsorbed enzyme changed due to the binding function of PHB depolymerase, as reported previously.[27,28] Figure 6B shows the PHB depolymerase molecules adsorbed on the PLLA/ata-PHB (50/50) blend thin film. In the PLLA domain as well as at the interface of the blends, PHB depolymerase molecules were observed to be similar to the PLLA thin film. Unexpectedly, the enzyme molecules were found on the surface of ata-PHB domain with higher molecular mobility. The number of the enzymes on the ata-PHB domain was smaller than that on the PLLA domain. This result indicates that PHB depolymerase can adsorb on both domains, while that mobile ata-PHB domain disturbs the adsorption function of PHB depolymerase.

Figure 7 shows the AFM phase images of PHB depolymerase adsorbed on PDLLA/ata-PHB (50/50) thin film. Darker and brighter regions correspond to PDLLA and ata-PHB domains,

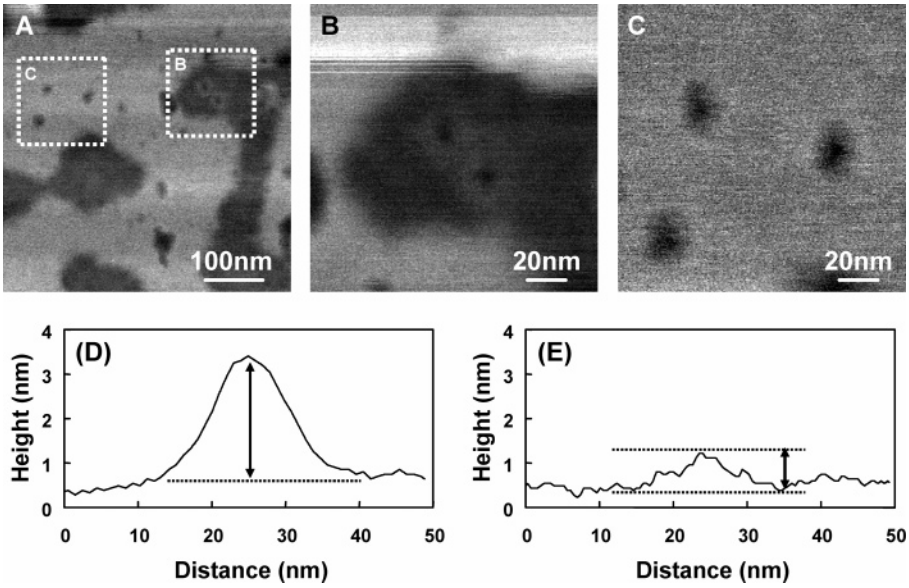

Figure 7. AFM phase images (A−C) of PDLLA/ata-PHB blends (50/50) after enzymatic treatment by PHB depolymerase for 5 min. Frames B and C are higher enlarged images in frame A. Frames D and E show the cross sectional data of PHB depolymerase molecule found on the PDLLA and ata-PHB domains, respectively.

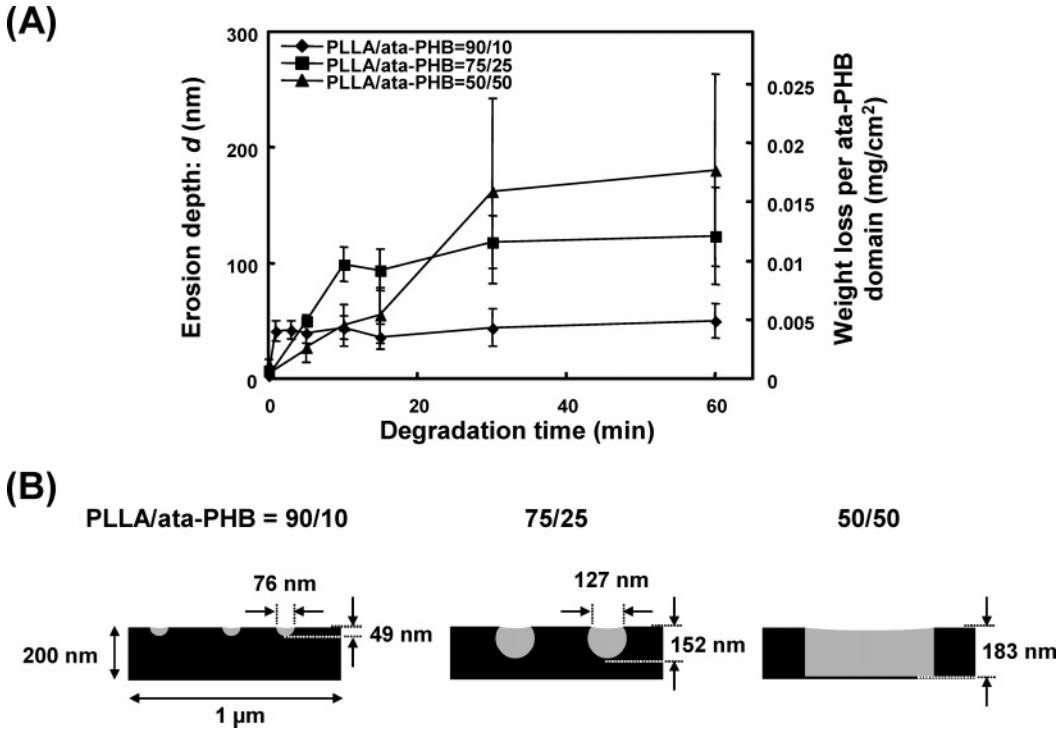

Figure 8. Erosion depth of ata-PHB domain in PLLA-400K/ata-PHB blend thin films measured from stepwise enzymatic degradation experiment by PHB depolymerase (frame A). Both erosion depth (*d*) of ata-PHB domain and weight loss/ata-PHB domain calculated from eq 1 were plotted as a function of degradation time for the blend thin films with different compositions.[41] Frame B shows the sectional view of domain structure based on the results in Table 3. The black part shows the PLLA domain, while the gray region indicates the ata-PHB domain.

respectively. As shown in Figure 7A, PHB depolymerase adsorbed on both domains. Figure 7B,C shows the higher enlarged image of Figure 7A. In terms of phase response, PDLLA around the adsorbed enzyme was changed to brighter contrast, while ata-PHB around the enzyme remained unchanged. A similar result was found in PLLA/ata-PHB blends of Figure 6. This result suggests that PHB depolymerase adsorption on PDLLA affects the surface property, while that on ata-PHB has less effect on changing the surface morphology. Figure 7D,E shows the cross-sectional data of PHB depolymerase molecules adsorbed on PDLLA and ata-PHB domains, respectively. The enzyme on the PDLLA was ca. 2.5 nm in height, while that on ata-PHB was almost buried from the polymer surface. This result indicates that surface properties under the binding enzyme strongly affect the adsorption behavior of PHB depolymerase.

Insight into Enzymatic Degradation Manner by PHB Depolymerase. It has been reported that PHB depolymerase is difficult to degrade ata-PHB chains due to the lower binding affinity, resulting in the lower function of catalytic domain.[29−31] From our present result, small numbers of PHB depolymerase can be adsorbed on ata-PHB domain, as shown in Figures 6 and 7. Therefore, we propose that PHB depolymerase on matrix domain (e.g., PLLA or PDLLA) mainly takes part in the

hydrolysis reaction of ata-PHB chains because the enzymes have relatively stable binding sites on the material surface. The enzymes also bound on the ata-PHB domain, but they appear to have difficulties accessing the catalytic domain of the enzyme to ata-PHB chains. The higher molecular mobility of the ata-PHB domain restricts the conformational changes of the enzyme for the hydrolysis reaction.

Such a proposal can be supported by the enzymatic degradation ratio of ata-PHB domain by PHB depolymerase. Figure 8A shows the erosion depth of ata-PHB domain in the PLLA/ata-PHB blend thin films measured from stepwise enzymatic degradation, which is the same method as Figure 5 and eq 1.[41] Note that Figure 5 is the erosion depth profile of PLLA domain during enzymatic degradation by proteinase K, whereas Figure 8A shows the erosion depth of ata-PHB domain by PHB depolymerase. Since the depth of ata-PHB domains is dependent on the blend ratio (Table 3 and Figure 8B), the erosion depth of Figure 8A was saturated at the depth of ata-PHB domain. Therefore, the initial erosion rate is important for the determination of degradation manners by PHB depolymerase without the effect of domain depth. In Figure 8A, the initial erosion rates were decreased in the following order: 90/10, 75/25, 50/50 blends. This tendency is related to each domain size of ata-PHB, which increased with increasing the ata-PHB composition (Table 3 and Figure 8B). Therefore, the initial erosion rate along the depth direction is suggested to be dependent on the domain size, i.e., perimeter of the domain. Adsorption of PHB depolymerase mainly took place on the PLLA domain in the blend thin film, whereas the minor adsorption occurs on ata-PHB domain, as shown in Figures 6 and 7. Therefore, smaller domain size is advantageous for the fast erosion along the depth direction because the enzyme molecules at the periphery of ata-PHB domain participate in the erosion process, and access the catalytic domain of the enzyme to ata-PHB chains.

Conclusions

Phase structure of PLA/ata-PHB blends was studied by AFM and enzymatic degradation treatment. The blends showed phase-separated morphologies due to the immiscibility. The domain morphologies of both components were assigned on the basis of the information from the compositional ratio, AFM phase response, and enzymatic degradation behavior.

The ata-PHB domains were eroded by PHB depolymerase, and the depth profile indicated that ata-PHB domains were distributed in the lateral as well as depth directions. In the case of proteinase K, PLLA domains were degraded. Stepwise enzymatic degradation experiment has revealed the heterogeneous degradation manner, and the erosion depth was limited below 50−100 nm for blend thin films of ca. 200 nm in thickness.

Direct AFM observation of PHB depolymerase molecules was performed to study the adsorption behavior of the enzymes on the surface of blend thin films. The enzyme molecules were found on both PLLA and ata-PHB domains. The number of enzyme molecules on the PLLA domain was much larger than that on the ata-PHB domain, suggesting that PHB depolymerase preferentially adsorbs on the PLLA domain rather than the ata-PHB domain. The initial erosion rate of ata-PHB domain was dependent on the domain size, suggesting that the enzyme molecules at the periphery of ata-PHB domain mainly participate in the erosion process.

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