Alkaline Hydrolysis of Solution-Grown Poly(L-lactic acid) Single Crystals

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Abstract: The changes in morphology and crystalline state of solution-grown single crystals of poly(L-lactic acid) (PLLA) in aqueous NaOH solution of various strengths were investigated by means of transmission electron microscopy, atomic force microscopy and gel permeation chromatography. The degradations of hexagonal and lozenge shaped PLLA single crystals started from the lateral side of the crystal to generate the notched morphology at initial stage of hydrolysis. As hydrolysis proceeded, the molecular weight of degraded PLLA crystals corresponded to equal the value calculated from lamellar thickness measured by atomic force microscopy, suggesting that the tight chain-folding region of PLLA molecules on crystal surface was degraded by alkaline hydrolysis. Based on the results, alkaline hydrolysis with NaOH solution firstly occurs at the loosely chain-packing region on crystal edges, and then gradually progresses from both crystal edges and tight chain-packing region with chain-folding on the crystal surface.

1. Introduction

In the chemical or enzymatic hydrolysis of biomaterials, the distribution of crystal regions, lamellar crystal size, and crystal morphology and structure may play a decisive role in the rate of hydrolysis. To elucidate the mechanism of enzymatic degradation on the crystal region, single crystals were prepared as model substrate, and their enzymatic degradations were studied. Enzymatic degradations using single crystals have been firstly performed on β(1→4)xylan single crystals with xylanases [1] and lamellar single crystals of nigerin with myceliastatinase [2]. These techniques were applied to the enzymatic degradation of poly[(R)-3-hydroxybutyrate] (P(3HB)) single crystals that are a biodegradable and biocompatible thermoplastic [3-6]. An edge attack model for enzymatic degradation of P(3HB) single crystals was also presented in the other aliphatic polyester single crystals of P(3HB) copolymers with different second monomer units [7,8] and PLLA [9].

In contrast, regarding the chemical degradation, completely different results have been reported. P(3HB) single crystals were degraded selectively from crystal surfaces with gaseous methanaline, followed by analysis of gel permeation chromatography of their degradation products [10]. This selective degradation was subsequently confirmed on the degradation of P(3HB) single crystals by methanaline and cyclohexylamine in solution, which was investigated by means of transmission electron microscopy and atomic force microscopy [11,12]. More recently, we reported the visualization of alkaline hydrolyses with NaOH solution of P(3HB) single crystals [13]. The degradation of lath-shaped single crystals progressed from both loosely chain-packing region on crystal edges and chain-folding region on crystal surface. These observations were further reported in the morphological study on hydrolytic degradation of poly(tetramethylene succinate) single crystals [14].

In this paper, we present the degradation mechanism of PLLA single crystals by alkaline hydrolyses with a dilute NaOH solution, using transmission electron microscopy, atomic force microscopy and gel permeation chromatography

2. Experimental Section

2.1 Preparation of Lamellar Single Crystals.

The poly(L-lactic acid) (PLLA, number-average molecular weight (Mn) = 83,000 and polydispersity (DPI) = 1.4) sample was purchased from Polysciences, Inc. (Warrington, PA). The PLLA sample was purified by the reprecipitation in methanol from chloroform solution and dried in vacuo for one week. 5 mg of PLLA was dissolved into 10 mL of p-xylene at 130 °C. The solution was kept there for 15 minutes, after which slow cooling was applied until 90 °C (-0.5 °C/min), and the solution was kept there for 24 hours. Slow cooling was applied by
cutting off the heating element of a silicone oil bath. The crystals were collected by centrifugation (3000 rpm) and washed three times with methanol at room temperature.

2.2 Alkaline hydrolysis.

For alkaline hydrolysis, the crystals were collected by centrifugation, washed once with distilled water, and resuspended in the given concentration of NaOH solution. Single crystals of PLLA were hydrolyzed in NaOH solution at 37 °C for various hours, which was not shaken in order to prevent the single crystals from breaking down by tremble. The degraded single crystals were washed three times with distilled water to remove the NaOH solution. The crystals were then redispersed in methanol, washed twice by centrifugation, and resuspended in methanol.

2.3 Transmission Electron Microscopy.

Drops of the crystal suspension before and after alkaline hydrolysis were deposited on carbon-coated grids, allowed to dry, and then shadowed with a Pt-Pd alloy. For electron diffraction purposes, the crystals were only allowed to dry. These grids were observed with a JEM-2000FX II electron microscope operated at an accelerating voltage of 120 kV for both electron diffraction and imaging of shadowed crystals. Electron diffraction diagrams and images were recorded on Kodak SO-163 and 4489 films, respectively, developed for 4 minutes with Kodak D19 developer (diluted in water 1/2, v/v).

2.4 Atomic Force Microscopy.

The thicknesses and surface morphology of single crystals before and after alkaline hydrolysis were investigated on the basis of Atomic Force Microscopy (AFM). AFM was performed with a SPI3700/SPA300 (Seiko Instruments Inc.). Pyramid-like Si$_3$N$_4$ tips, mounted on 100 μm long micro cantilevers with spring constants of 0.09 N/m were applied for the contact mode experiments. Simultaneous registration was performed in the contact mode for height and deflection images. Drops of the crystal suspension before and after alkaline hydrolysis were given on mica and allowed to dry. All images were recorded at room temperature.

2.5 Molecular Weight Measurement.

All molecular weight data of single crystals before and after alkaline hydrolysis were obtained by gel permeation chromatography (GPC) at 40 °C. using a Shimadzu 10A GPC system and 6A refractive index detector with joint columns of Shodex K-80M and K-802 (each 4.6 x 300 mm). Chloroform was used as eluent at a flow rate of 0.8 mL/min, and a sample concentration of 1.0 mg/mL was employed. The number-average and weight-average molecular weights (Mn and Mw) were calculated by using a Shimadzu Chromatopac C-R7A plus equipped with a GPC program. Molecular weight was obtained with polystyrene standards of low polydispersities.

3. Results and Discussion

3.1 Morphological Changes of Lamellar Crystals.

Typical electron micrographs of lozenge and hexagonal shaped PLLA crystals with screw dislocations grown from a dilute solution of p-xylene are shown in Figure 1. The crystals comprising stacks of lamellae have variable thickness; some are transparent to the electron beam whereas others are completely opaque. Each monolamellar part at the edge of all PLLA crystals yields the well-resolved electron diffractogram.

![Electronmicrographs of PLLA lamellar crystals grown from a dilute solution of p-xylene. (A) lozenge shaped and (B) hexagonal shaped.](image)

On the basis of the orthogonal unit cell in PLLA crystals reported previously [15-18], the lozenge shaped crystals could be considered to occur with [110] as
growth planes, like a flat lamellar polyethylene crystal [19]. Compared with the smooth faces well-defined by \{110\} of lozenge shaped crystal, the growth faces of hexagonal shaped crystal except \{110\} planes are slightly rough. If PLLA crystallized as perfect hexagons, the growth rate should be the same in all planes. This contradiction suggests that the hexagonal crystal has pseudo-hexagonal symmetry but actual orthogonal packing of PLLA molecules.

Fig. 2 AFM images of PLLA single crystals; (A) hexagonal shaped and (B) lozenge shaped. Inset: line profile data of white lines.

The thickness by AFM of the monolamellar part of lozenge shaped crystals yielded the values of 9-10 nm, while hexagonal shaped crystals had a thickness of 11-12 nm as shown in Figure 2. These results corresponded to those discerned from the length of the shadow in electron micrographs reported by Kalb and Pennings [17]. Taking into consideration the fiber repeat distance and molecular weights, the chain foldings occur at lamellar surfaces of single crystals as with polyethylene [19], polypropylene [20], poly([R]-3-hydroxybutyrate) [11,12] and poly([R]-3-hydroxyvalerate) [21].

Fig. 3 Electron micrographs of PLLA single crystals after alkaline hydrolysis (A) after 48 h in 0.01 N NaOH solution; (B) after 3 h in 0.1 N NaOH solution. Arrows indicate diamond portions.

Figure 3 shows electron micrographs of PLLA lamellar crystals degraded in NaOH solutions of 0.01N for 48 h and 0.1N for 3 hour. The lateral sides of crystals treated with 0.01N-NaOH for 48 h were cut into notched-shapes, in spite of the whole outline retaining the original-shapes. Furthermore, some small fragments which seemed to be separated from the lateral sides of lamellar crystals were observed. On the other hand, in the case of 0.1N-NaOH treatment for 3h, as the degradation proceeded, a coarsed shape turned to be round and the crystal size decreased. In addition, one sees the diamond-shaped portions of the crystal separated from the remainder were detected as shown in Figure 3B. This diamond-shaped hole has been also observed in PLLA single crystals after enzymatic degradation by Proteinas K [9]. However, in our study these diamond-shaped holes could not be observed in the single crystals before enzymatic degradation, indicating that those holes were resulted from alkaline or enzymatic attacks. Thus, the
single crystals were selectively degraded from disordered molecular packing region with faster degradation rate. Electron diffractogram of rounded-shaped crystal inset in Figure 3B showed broad reflections and lower resolution than that of undegraded crystals, suggesting that molecular packing into the crystal lattice became slightly disordered due to the chain rotation around the fiber axis.

![Image of PLLA crystals after alkaline hydrolysis](image)

**Fig. 4** AFM images of PLLA crystals after alkaline hydrolysis; (A) lozenge shaped and (B) hexagonal shaped. Inset: line profile data.

Atomic force microscopy images of PLLA lamellar crystals after alkaline hydrolysis by a 0.1N-NaOH solution for 3 h and line profile data are shown in Figure 4. The lamellar thickness of lozenge shaped crystals decreased to 7 nm. Furthermore, in the case of hexagonal shaped crystals, center part of screw dislocation was degraded remarkably and lamellar thickness decreased as a whole. These results indicate that the single crystals are degraded from the crystal surface in addition to lateral sides of crystals, suggesting that the hydrolysis occurred at the chain-folding on the crystal surface.

### 3.2 Molecular Weight Changes

The measurement of molecular weight during the hydrolysis of single crystals can aid to elucidate the degradation mechanism of the molecular level. Partial degradation at the chain-folding surface of single crystals has been confirmed to cause a molecular weight change. Figure 5 shows the molecular weight distributions of PLLA obtained by gel permeation chromatography before and after degradation of PLLA single crystals by a NaOH solution for various strengths and lengths of time. At early stages of degradation, the molecular weight decreased and the distribution increased by a heterogeneous hydrolysis correlating to the degradation of crystal edges and crystal surface. These phenomena exhibit discrete peaks which become more prominent as the degradation proceeds. In the case of strong alkaline-hydrolysis (0.1N-NaOH), two main peaks remained at different molecular weights of 2,900 and lower than this above peak. Taking the fiber repeat distance of 2.78 nm with 10/3 screw symmetry along the molecular axis into consideration [15-18], these two molecular weights correspond to monolamellar thickness or less of crystals. Thus, the results further indicate that alkaline hydrolysis with NaOH solution occurred at the chain-folding surface of crystal.

![Molecular weight profiles](image)

**Fig. 5** Gel permeation chromatography profiles of PLLA single crystals before and after partial degradation in NaOH solution.

Results from the transmission electron microscopic and atomic force microscopic observations combined with molecular weight measurement indicated that solution-grown PLLA single crystals were completely degraded from both crystal edges and crystal surfaces by alkaline hydrolysis. Single crystals of aliphatic polyesters, grown from a dilute solution by isothermal crystallization, normally crystallize with chain-folding on crystal surface. The decrease of molecular weight measured by GPC and surface erosion confirmed by AFM of degraded single
crystals supported that the alkaline hydrolysis occurred from chain-folding of crystal surfaces. Schematic representation on the alkaline hydrolysis of PLLA single crystals in NaOH solution is shown in Figure 6.

![Figure 6](image-url)

**Fig. 6** Schematic representation on the alkaline hydrolysis of PLLA single crystals in NaOH solution: (A) degradation for switchboard model region with random reentry fold and central portion, and (B) surface and edge degradation.

The surface degradation of P(3HB) single crystals, with gaseous methylamine, methylamine in solution and cyclohexylamine in solution, has been already reported by Welland et al. [10] and Sykes et al. [11,12]. In the cases of methylamine in gaseous phase or in solution, the degradation yielded fragments with lengths corresponding to integer multiples of lamellar thickness. Iwata et al. reported the alkaline hydrolysis of P(3HB) single crystals with NaOH solution occurred from both crystal surface and crystal edges [13]. On the other hand, since cyclohexylamine is a bigger molecule than methylamine, the reduction of molecular weight by cyclohexylamine was more gradual and it was harder to attack the tight chain-folding [10]. When applied the enzymatic degradation of PLLA single crystals by a Proteinase-K (from *Tritirachium album*), the crystals were degraded only from crystal edges without decrease of molecular weight and lamellar thickness [9]. In this present study, NaOH is a quite small molecule and is probably able to attack tight chain-folding regions at the crystal surfaces.

### 4. Conclusions

In this paper we have reported the visualization of alkaline hydrolysis with NaOH solution of PLLA single crystals grown from *p*-xylene by means of transmission electron microscopy and atomic force microscopy. The degradation of hexagonal and lozenge shaped single crystals progressed from loosely chain-packing region on crystal edges, and as a result the lateral sides of crystals were cut into notched-shapes. The notched-shaped crystals were further hydrolyzed from both crystal edges and crystal surfaces to yield rounded crystals with diamond-shaped portions of the crystal separated from the remainder. The molecular weight of remaining crystals decreased with time and the values of stable molecular weight corresponded to thickness less than crystal lamellae. These results indicate that alkaline hydrolysis in NaOH solution occurs at the tight chain-folding on the crystal surface and in the lamellar interior.

### References