# Alkaline Hydrolysis of Enantiomeric Poly(lactide)s Stereocomplex Deposited on Solid Substrates

**Takeshi Serizawa,† Yuuya Arikawa,† Ken-ichi Hamada,† Hiroo Yamashita,† Tomoko Fujiwara,‡ Yoshiharu Kimura,‡ and Mitsuru Akashi*,†**

*Department of Nanostructured and Advanced Materials, Graduate School of Science and Engineering, Kagoshima University, Korimoto 1-21-40, Kagoshima 890-0065, Japan, and Department of Polymer Science and Engineering, Faculty of Textile Science, Kyoto Institute of Technology, Goshokaido-Cho, Matsugasaki, Sakyo-Ku, Kyoto 606-8585, Japan*

*Received October 23, 2002*
*Revised Manuscript Received December 16, 2002*

Regulated assemblies and crystallization of polymers having a well-defined nanostructure are attractive not only for expanding the functional capabilities of the polymers but also for investigating novel characteristics of the polymers assembled. The formation of racemic crystals (so-called stereocomplex) between poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) with 1:1 stoichiometry, in which both polymers are alternately packed with $\beta$-form $3_1$-helices of the opposite absolute configuration (left- and right-handed, respectively) side by side with van der Waals contact, has been previously investigated.[1] Since poly(lactide)s (PLAs) can be hydrolyzed to obtain lactic acid under biological conditions, differences in the hydrolytic activities between the stereocomplex and homogeneous crystals with α-form $10_3$-helices were of interest for potential biomedical applications. Li and Vert demonstrated that stereocomplexes were formed during hydrolysis of poly(L-lactide-*co*-D-lactide) films (size 10 × 10 × 2 mm) in a phosphate buffer solution, resulting in greater hydrolysis of relatively random monomer unit sequences, which did not join stereocomplexes.[2] Tsuji also demonstrated that the stereocomplex film (size 1.8 mm × 3.0 mm × 50 mm or 25 $\mu$m) was stable in a phosphate buffer solution, and its hydrolytic rate was less than that of homogeneous PLA crystals.[3] Since it is difficult to obtain highly crystalline stereocomplex films, the difference in hydrolytic properties between the stereocomplex and the homocrystals of PLAs has not been conclusively demonstrated. Although the previous studies discussed the hydrolysis of PLAs using thick specimens, the difference might be potentially demonstrated using ultrathin assemblies with a regularly conformational structure.

Layer-by-layer (LbL) assembly promises stepwise deposition of interactive polymers having cationic and anionic charges to produce ultrathin polymer films by alternate immersion of substrates into the polymer solutions.[4] Not only electrostatic interactions but also other interactions (hydrogen bounds, charge transfer, and van der Waals) have been utilized for LbL assembly.[5] We have applied stereocomplex formation of enantiomeric PLAs to LbL assembly to produce regularly conformational ultrathin polymer films.[6] Quantitative detection of amounts assembled and the presence of a melting point at approximately 230 °C provided evidence of complex formation. Since the LbL assembly of the PLAs involved monolayer deposition of the interacting polymer with the polymer already deposited at each step, almost all of the polymers potentially participated in the complex, indicating a macromolecularly regulated structure. Furthermore, PLLA and PDLA can be epitaxially grown on the outermost surface of PDLA and PLLA, respectively, to produce heterogeneous assemblies, when the stereocomplex films were immersed in solutions of each enantiomer.[6] Accordingly, these assemblies might facilitate study of the hydrolytic properties of the PLA stereocomplex.

In the present study, PLA stereocomplexes prepared by the stepwise LbL assembly of PLLA and PDLA from their acetonitrile solutions on a solid substrate were hydrolyzed under concentrated alkaline conditions as an acceleration test. The assembly and hydrolysis processes were quantitatively analyzed using a quartz crystal microbalance (QCM) as the gold substrate. The complex hydrolyzes faster than crystals of each constituent enantiomer.

PLLA and PDLA with average number molecular weights ($M_n$) of 27 700 and 30 800 and distributions ($M_w/M_n$) ($M_w$: average weight molecular weights) of 1.51 and 1.55, respectively, were prepared following methods in previous studies.[7] An AT-cut quartz crystal with a parent frequency of 9 MHz was obtained from USI (Japan). The crystal (9 mm in diameter) was coated on both sides with gold electrodes 4.5 mm in diameter. The PLA stereocomplex has been previously deposited on a QCM silver electrode, as reported.[6] To obtain a QCM with a smoother surface, a QCM with a gold electrode and a mean roughness of 1.8 nm was applied. Enantiomeric PLAs were deposited from their acetonitrile solutions at concentrations of 10 mg mL$^{-1}$ at 50 °C by alternate immersion of the QCM for 15 min in each solution. After rinsing with acetonitrile and drying with $N_2$ gas at each deposition step, a frequency counter (Iwatsu model SC7201) analyzed the frequency decrease of the QCM ($\Delta F$) to estimate the amount of polymer deposited ($\Delta m$) from Sauerbrey's equation[8]

$$-\Delta F = \frac{2{F_0}^2}{A\sqrt{\rho_q \mu_q}} \Delta m$$

where $F_0$ is the parent frequency of the QCM (9 × 10$^6$ Hz), $A$ is the electrode area (0.159 cm$^2$), $\rho_q$ is the density of the quartz (2.65 g cm$^{-3}$), and $\mu_q$ is the shear modulus (2.95 × 10$^{11}$ dyn cm$^{-2}$). The present assembly started with PLLA; however, the starting polymer did not affect the assembly process. The deposited or spin-coated amount of PLA was approximately −4000 Hz/QCM, which corresponded to 3500 ng/electrode (11 $\mu$g cm$^{-2}$). It took approximately 42 LbL steps for the assembly of the appropriate amount. On the other hand, PLLA was grown for 12 h on the 16-step LbL assembly with a PDLA surface to deposit the given amount. In addition, spin-coated films of PLAs were also prepared by the conventional method. For crystallization of the film, the spin-coated film was annealed at 200 °C for 10 min and then gradually cooled at ambient temperature in the

† Kagoshima University.
‡ Kyoto Institute of Technology.

10.1021/ma021611+ CCC: $25.00 
Published on Web 02/19/2003

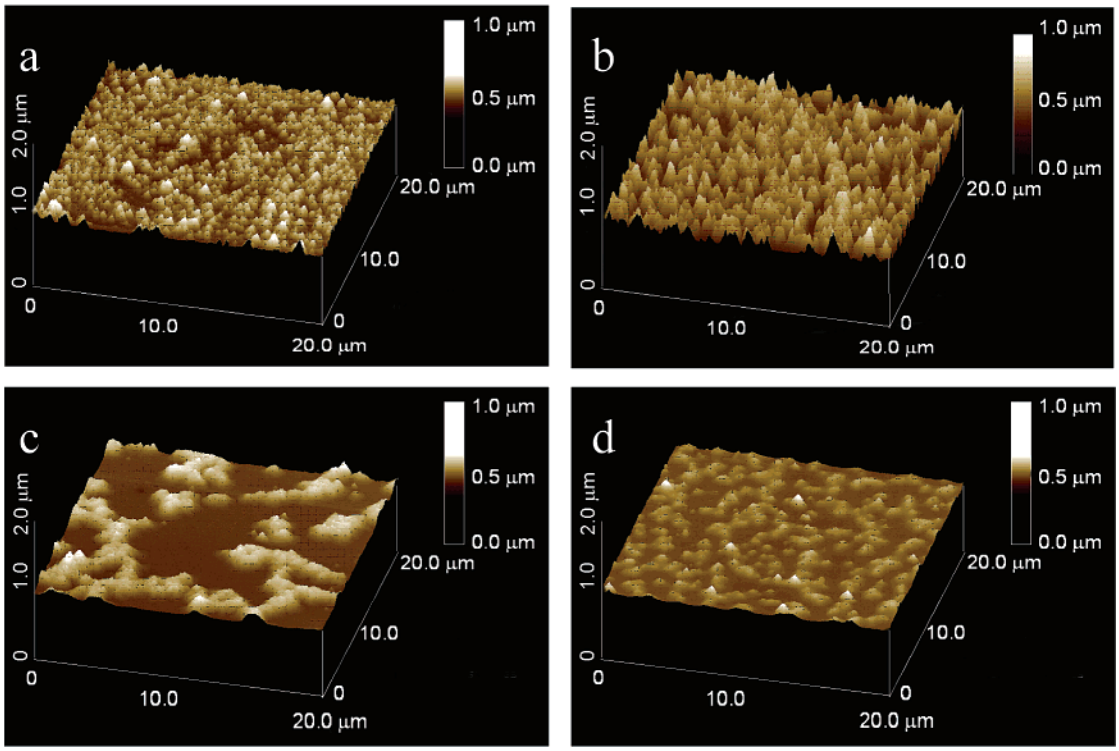


**Figure 1.** AFM images of (a) a 42-step LbL assembly of enantiomeric PLAs, (b) the heterogeneous assembly (12-step LbL assembly and subsequent growth of PLLA), (c) crystallized PLLA, and (d) amorphous PLLA on a QCM substrate.

oven. To prepare amorphous films, the annealed film was rapidly cooled at 0 °C. When we assume the molecularly smooth assemblies and their densities to be 1 g $cm^{-3}$, the apparent thickness of these films was approximately 90 nm based on the frequency decrease. Atomic force microscopic (AFM) images were obtained using a Digital Instruments NanoScope III that was operated in a tapping mode in air at ambient temperature. We did not perform any image processing other than flat leveling. The mean roughness (*Ra*) in a given area of observation was estimated from the following equation:

$$Ra = \frac{1}{Lx Ly} \int_0^{Ly} \int_0^{Lx} |F(x,y)| \, dx \, dy$$

where $F(x,y)$ is the surface relative to the center plane, which is a flat plane parallel to the mean plane, and $Lx$ and $Ly$ are the dimensions of the surface.

To analyze whether PLA stereocomplexes can be deposited on a QCM with a gold electrode, the stepwise shift of frequency was analyzed by alternate immersion of a QCM into acetonitrile solutions of PLLA and PDDA (original QCM data not shown). The ratio of total amounts between PLLA and PDLA assembled (PLLA/PDLA) was 0.94, which was consistent with the value estimated from bulk stereocomplex analyses (PLLA/PDLA = 1). A profile of the frequency shift against step number showed an exponential curve. AFM observation revealed the surface topology to be a dotted structure with a mean roughness of 33 nm (Figure 1a). These observations are similar to those obtained from assembly onto a QCM with a silver electrode,[6] indicating stepwise formation of stereocomplex on the present QCM surface. The dot size is apparently greater than a macromolecular size. The PLAs possibly rearranged to form greater aggregates after stereocomplex formation on the surface. Heterogeneous LbL assembly of enantiomeric PLAs was also performed. The PLLA deposition for 12 h after the 16-step LbL assembly with a PDLA surface resulted in deposition of approximately −4000 Hz (11 $\mu$g $cm^{-2}$ deposition). The AFM image indicated a different assembly with a mean roughness of 73 nm (Figure 1b) compared to the LbL assembly (Figure 1a). The AFM images of spin-coated films of PLLA in crystalline (Figure 1c) and amorphous (Figure 1d) states were also obtained and revealed different surface topologies. It is necessary to consider the surface area of the assemblies in order to analyze the hydrolytic properties. Percent increments of surface areas of Figure 1a−d, which derived from the formation of dotted structures on the substrate surface, were 8.5, 16, 1.1, and 0.6%, respectively. The individual surface areas of the films were estimated by using the percent increments. Accordingly, the surface areas normalized the following hydrolytic data.

Figure 2 shows the remaining weight of the assemblies and spin-coated films on the QCM surface against time, when the QCM was immersed in an aqueous 0.01 N NaOH solution at 37 °C. Each plot was ex situ monitored by measuring the frequency in air after rinsing with water and drying. The profiles of spin-coated films of PDLA were the same as those of PLLA, since alkaline hydrolysis did not differ between enantiomeric PLAs. The initial hydrolytic rates normalized by the individual surface areas are summarized in Table 1. The profiles clearly indicated faster hydrolysis of the LbL assembly (Figure 2a). The rate of hydrolysis of the LbL assembly was more than 7 times greater than that of the crystallized film (Figure 2c) and was equivalent to that of the amorphous film (Figure 2d), although the PLLA in the amorphous film may be partially crystallized during the cooling process. The PLAs form $\beta$-form $3_1$-helices and α-form $10_3$-helices in the stereocomplexes

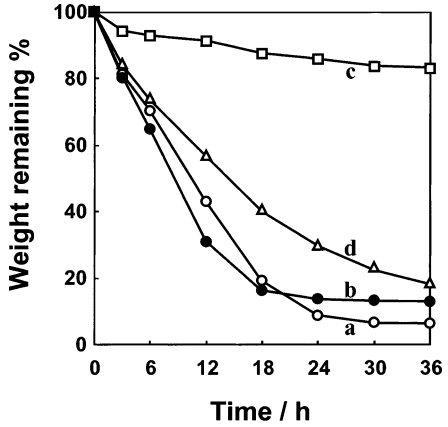


**Figure 2.** Alkaline hydrolysis of (a) the LbL assembly, (b) the heterogeneous assembly, (c) crystallized PLLA, and (d) amorphous PLLA.

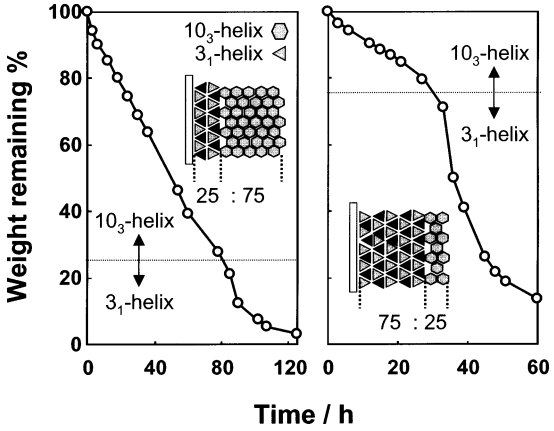


**Figure 3.** Alkaline hydrolysis of assemblies comprised of an inner layer of stereocomplex and outer PLLA crystal layers.

**Table 1. Initial Hydrolysis Rates of Various PLA Films**[a]

| films[b] | hydrolysis rates (weight loss % $h^{-1}$ $cm^{-2}$) |
|---|---|
| stereocomplex PLAs | 11.7 ± 0.9 |
| heterogeneous PLAs[c] | 13.9 ± 0.1 |
| crystallized PLLA | 1.9 ± 0.3 |
| amorphous PLLA | 10.8 ± 0.4 |

[a] The rates were estimated from a slope between 3 and 6 h of the hydrolysis profiles and then normalized by the individual surface areas. [b] The amounts of PLAs assembled were approximately −4000 Hz (11 $\mu$g $cm^{-1}$) in all cases. [c] The assembly of the initial stereocomplex layer (−1000 Hz) and the subsequent deposition of PLLA (−3000 Hz).

and in the crystals, respectively. This difference in crystalline structure governs the alkaline hydrolysis of PLAs. In other words, the $3_1$-helices might be more readily hydrolyzed in alkaline conditions compared to the $10_3$-helices. Furthermore, the heterogeneous assembly was hydrolyzed at a rate similar to that of the LbL assembly (Figure 2b). Analysis revealed that the PLLA was epitaxially grown on the LbL assembly possibly forming $3_1$-helices,[6] suggesting greater hydrolysis of the $3_1$-helices. On the other hand, a certain percentage of the polymer usually remained even after lengthy immersion in NaOH. This is possibly due to direct influence of the gold substrate, and the polymers directly adsorbed on the gold substrate seemed not to be hydrolyzed or desorbed under the present conditions. When PDLA with a greater molecular weight ($M_n$ 69 000) was used for the LbL assembly, the hydrolytic rate decreased slightly, indicating that desorption of PLAs can be controlled by the molecular weight.

It was possible to prepare assemblies comprised of the initial stereocomplex assembly and then spin-coated with an outer layer of PLLA. The assembly was thermally treated for 10 min at 200 °C, at which the spin-coated PLLA melted and the stereocomplex did not melt, and then slowly cooled, to obtain an assembly having two layers comprised of a stereocomplex under layer and a crystallized outer PLLA layer. The hydrolysis profile of these assemblies can be divided into two steps: the initial slow hydrolysis of the PLLA crystals and then the rapid hydrolysis of the remaining stereocomplex (Figure 3). The ratio of PLAs deposited as stereocomplexes and homocrystals could be altered to obtain different hydrolytic profiles. These observations suggest that PLA was hydrolyzed from the surface of the assembly and that the present process may be used to prepare stepwise hydrolyzable ultrathin PLA films with variable crystal structures ($10_3$- or $3_1$-helix) on material surfaces. Regulation of the layer structure as well as the crystal structure in turn permits control of the hydrolytic rate.

Alkaline hydrolysis of stereocomplexes of enantiomeric PLAs prepared by LbL assembly was quantitatively analyzed using a QCM substrate. The stereocomplex was readily hydrolyzed compared to crystalline PLAs, and the hydrolytic rate was similar to that of amorphous PLAs, even though there was a slight difference in crystal structure between the complex and the homocrystals. This is the first report of the greater rate of hydrolysis of PLA stereocomplexes. Manipulation of the layer structure within the assemblies also permits regulation of the hydrolysis rate. A variety of enzymes hydrolyze PLLA, and further research regarding enzymatic hydrolysis is now in progress.

**Acknowledgment.** This work was financially supported in part by Grant-in-Aid for Scientific Research in the Priority Area of "Molecular Synchronization for Design of New Materials System" (No. 13022258) and by Grant-in-Aid for Scientific Researches (No. 851/14780643) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

## References and Notes

(1) (a) Ikada, Y.; Jamshidi, K.; Tsujii, H.; Hyon, S.-H. *Macromolecules* **1987**, *20*, 906. (b) Tsujii, H.; Horii, F.; Hyon, S.-H.; Ikada, Y. *Macromolecules* **1991**, *24*, 2719. (c) Tsujii, H.; Hyon, S.-H.; Ikada, Y. *Macromolecules* **1991**, *24*, 5651. (d) Tsujii, H.; Hyon, S.-H.; Ikada, Y. *Macromolecules* **1991**, *24*, 5657. (e) Tsujii, H.; Hyon, S.-H.; Ikada, Y. *Macromolecules* **1992**, *25*, 2940. (f) Tsujii, H.; Ikada, Y. *Macromolecules* **1992**, *25*, 5719. (g) Tsujii, H.; Horii, F.; Nakagawa, M.; Ikada, Y.; Odani, H.; Kitamaru, R. *Macromolecules* **1992**, *25*, 4114. (h) Tsujii, H.; Ikada, Y. *Macromolecules* **1993**, *26*, 6918. (i) Brizzolara, D.; Cantow, H.-J.; Diederichs, K.; Keller, E.; Domb, A. J. *Macromolecules* **1996**, *29*, 191.
(2) Li, S. M.; Vert, M. *Macromolecules* **1994**, *27*, 3107.
(3) Tsuji, H. *Polymer* **2000**, *41*, 3621.
(4) (a) Decher, G. *Science* **1997**, *277*, 1232. (b) Lvov, Y.; Möhwald, H. *Protein Architecture: Interfacing Molecular Assemblies and Immobilization Biotechnology*; Dekker: New York, 2000. (c) Bertrand, P.; Jonas, A.; Laschewsky, A.; Legras, R. *Macromol. Rapid Commun.* **2000**, *21*, 319. (d) Tripathy, S., Kumar, J., Nalwa, H. S., Eds.; *Handbook of Polyelectrolytes and Their Applications*; American Scientific Publishers: Los Angeles, 2002; Vol. 1.

(5) (a) Stockton, W. B.; Rubner, M. F. *Macromolecules* **1997**, *30*, 2717. (b) Sukhishvili, S. A.; Granick, S. *J. Am. Chem. Soc.* **2000**, *122*, 9550. (c) Wang, L.; Cui, S.; Wang, Z.; Zhang, X. *Langmuir* **2000**, *16*, 10490. (d) Hao, E.; Lian, T. *Chem. Mater.* **2000**, *12*, 3392. (e) Shimazaki, Y.; Mitsuishi, M.; Ito, S.; Yamamoto, M. *Langmuir* **1997**, *13*, 1385. (f) Serizawa, T.; Hamada, K.-I.; Kitayama, T.; Fujimoto, N.; Hatada, K.; Akashi, M. *J. Am. Chem. Soc.* **2000**, *122*, 1891.

(6) Serizawa, T.; Yamashita, H.; Fujiwara, T.; Kimura, Y.; Akashi, M. *Macromolecules* **2001**, *34*, 1996.

(7) For example: Amass, W.; Amass, A.; Tighe, B. *Polym. Int.* **1998**, *47*, 89.

(8) Sauerbrey, G. *Z. Phys.* **1959**, *155*, 206.

MA021611+