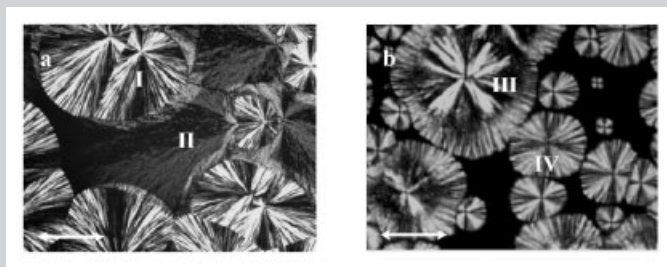


Summary: A new crystal morphology (δ form) of poly(3-hydroxypropionate) (PHP) is found in cast and melt-crystallized PHPs with low molecular weight, in which the PHP chains possibly adopt a 2_1 helix rather than the *trans* conformation found in the β or γ form. The fusion

temperature- and the crystallization temperature-dependent polymorphism are responsible for the dual morphologies and the unique growth kinetics of spherulites in the melt-crystallized PHPs.



a) A dual-morphology developed at 70 °C in PHP films after melting at 117 °C and b) that formed during cooling at a rate of 1 °C · min⁻¹ from 130 °C.

A New Crystal Form, Polymorphism, and Multi-Morphology in Biodegradable Poly(3-hydroxypropionate)

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Introduction

Biodegradable poly(hydroxyalkanoate)s (PHAs) can be hydrolyzed by the attack of enzymes.^[1] It is well known that solid-state properties, that is, crystallinity^[2] and crystal thickness,^[3] are inherent factors that affect the enzymatic degradation behavior of PHAs. In addition, it has also been speculated that the crystalline structure would regulate the biodegradability of PHAs. However, the polymorphism of biodegradable PHAs has not received the attention it deserves so far. Only limited research has been reported to date, on the crystal modifications in poly([R]-3-hydroxybutyrate) (PHB),^[4] poly(3-hydroxypropionate) (PHP),^[5–8] and poly(L-lactide).^[9] And few systematic investigations have

been carried out to determine the polymorphism of PHAs. The poor understanding of the polymorphic behavior of PHAs prevents us from clarifying the effect of polymorphism on biodegradation, which has recently been of some concern to biologists and polymer scientists.

Poly(3-hydroxyalkanoate)s (P3HAs) are based on a 3-hydroxypropionate (HP) skeleton in terms of chemical structure. Their conformations in the crystalline state are known to depend on the HP skeleton. The conformational analysis predicts that almost all PHAs will have identical conformations in the crystalline state, that is, a 2_1 helix and/or *trans* conformation.^[10] Because of the similarity in the chemical structure and the conformation in the crystalline state, research into the polymorphism of PHP may possibly

shed light on the understanding of the crystallization and the biodegradation of PHAs.

It is well known that the PHP backbones can be regularized into a 2_1 helix conformation in the α form (a fiber repeat distance of $c = 0.702$ nm),^[6,7] the *trans* conformation in the β form^[5–7] by drawing the cast films at several temperatures, or the *trans* conformation in the solution-grown γ -form lamellar.^[8] The orthorhombic unit cell of the β form has the dimensions of $a = 0.773$ nm, $b = 0.448$ nm, and $c = 0.477$ nm,^[5] while the two-chain, C-faced, orthorhombic unit cell of the γ form has the dimensions of $a = 0.700$ nm, $b = 0.490$ nm, and $c = 0.493$ nm.^[8] The crystalline structure of the α form is still unclear to date.

Till now, the polymorphism of PHP and the driving force leading to the polymorphism have not been fully understood yet. In this paper, the polymorphic behavior of PHP is investigated by employing X-ray diffraction, FT-IR spectroscopy, and polarized optical microscopy. It is found that three crystal modifications, that is, β , γ , and especially a new δ form can be formed by the melt-crystallization of PHPs free from strain. The crystalline structure of cast PHPs presents an unusual dependence on the molecular weight, and the multi-morphologies and the unique growth kinetics of spherulites are inherently correlated with the polymorphism of PHP.

Experimental Part

Materials

Three chemosynthesized PHPs (PHP18k: $\bar{M}_n = 1.8 \times 10^4$, $\bar{M}_w/\bar{M}_n = 2.48$; PHP56K: $\bar{M}_n = 5.6 \times 10^4$, $\bar{M}_w/\bar{M}_n = 2.17$; PHP70k: $\bar{M}_n = 7.0 \times 10^4$, $\bar{M}_w/\bar{M}_n = 2.03$) were prepared by a ring-opening polymerization of propiolactone and purified from solution in chloroform by precipitation in heptane.

Preparation of Samples

Melt-crystallized PHPs were compression molded at a chosen temperature (110, 117, or 130 °C) for 1.5 min under a pressure of 5 MPa, using a Toyoseiki Mini Test Press-10. After compression molding, the PHPs were annealed for 1.5 min, and then quenched to a desired temperature of crystallization (T_c) (30 or 70 °C) for crystallization over 24 h. Cast PHPs were prepared by casting a solution of PHPs in chloroform with an appropriate concentration, 10 mg · mL⁻¹, on Teflon dishes. The solvent was allowed to evaporate slowly at 30 °C.

Analytical Procedures

Wide-angle X-ray diffraction (WAXD) measurements were carried out on a Rigaku RINT-2000 (Rigaku Co., Tokyo, Japan), working at 40 kV and 200 mA, with Ni-filtered Cu K α radiation ($\lambda = 0.15418$ nm). Scans were made between Bragg angles of 5–60° at a scanning rate of 1° · min⁻¹.

FT-IR spectra were determined on an AIM-8800 (Shimadzu Co., Japan), an automatic infrared microscope. All the spectra

were recorded under a given temperature at a resolution of 2 cm⁻¹ and with an accumulation of 64 scans.

The spherulite growth was followed by an Olympus BX90 polarizing microscope (Olympus Co., Tokyo, Japan) with a dry nitrogen gas purge. Microphotographs were recorded with a FUJIX Digital Camera HC-2500 3CCD.

Results and Discussion

X-Ray Diffraction

X-ray diffraction profiles of three cast PHPs with various molecular weights (MWs) are shown in Figure 1a. Three characteristic diffraction peaks with Bragg angles of 22.1, 25.4, and 31.5° indicate the presence of only the γ form in the cast PHP70Ks.^[7,8] In the X-ray diffraction profile of PHP56ks, the three characteristic diffraction peaks of the γ form still remain but are weakened. In addition, three new diffraction peaks can be clearly observed at 20.7, 23.3, and 30.0°, but any of them could not be assigned to the known α , β or γ form of PHP.^[5–8] With further lowering of the MW of PHP to 1.8×10^5 , only these unknown diffraction peaks remain and are further enhanced. Obviously, these diffraction peaks could arise from an unknown crystal

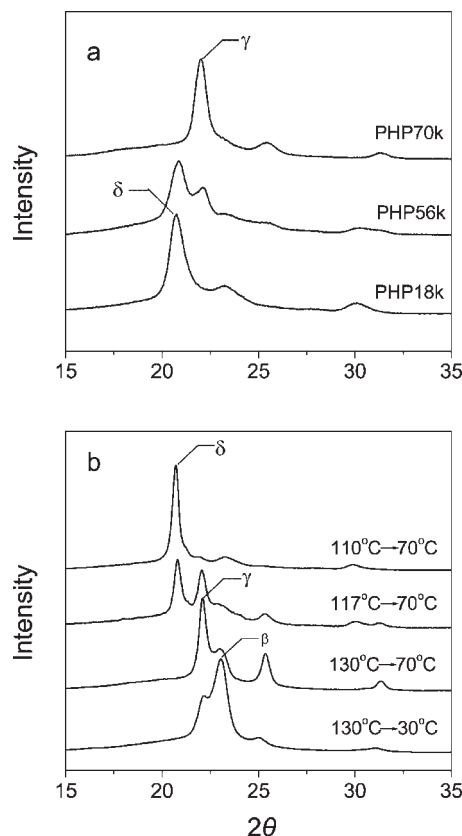


Figure 1. a) X-ray diffraction profiles of the cast PHPs with various MWs; b) X-ray diffraction profiles of melt-crystallized PHP18ks. The samples were first melted at a fusion temperature of 110, 117 or 130 °C for 3 min and then quenched to the crystallization temperature of 70 or 30 °C.

modification, which is referred to as the δ form by us from now on. Therefore, it seems that a low MW and a high MW favor the growth of the δ form and the γ form in the cast PHPs, respectively.

In Figure 1b are X-ray diffraction profiles of PHPs prepared by the melt crystallization of the cast PHP18ks. At the same crystallization temperature (T_c) of 70 °C, the crystalline structure greatly depends on the fusion temperature (T_f). Overwhelmingly, the δ form can be detected in the PHP samples melted at $T_f = 110$ °C and crystallized at 70 °C. Upon increasing the T_f to 117 °C, the γ form is found to co-crystallize with the δ form. Namely, the content ratio of the γ form to the δ form increases with increasing T_f in the temperature region of 110–130 °C. It is noted that not a pure γ form but a γ -rich phase is found in PHPs after melting at 130 °C, which contains a small amount of the β form, as indicated by the diffraction peak at 22.9 °C.^[5–7] Obviously, a lower T_f benefits the formation of the δ form, indicating that the growth of the δ form is related to the residual thermal history. Moreover, besides T_f , T_c also regulates the polymorphic behavior of PHP. As shown in Figure 1b, after melting at $T_f = 130$ °C, the PHP crystallized at 70 °C and that at 30 °C have, respectively, a γ -rich and a β -rich phase. It indicates that a low T_c and a high T_c favor the formation of the β form and the γ form, respectively. As opposed to the T_f -dependent polymorphism, the T_c -dependent polymorphism possibly originates from the difference in thermal accessibility between the γ and β form.

FT-IR Spectroscopy

In Figure 2 are shown the FT-IR spectrum in the 750–1450 cm^{-1} region of the melt-crystallized PHPs. The spectrum of the amorphous state is included for comparison, which was measured within the early stage of the crystallization induction at 70 °C.

Six absorption bands of the PHP β or γ form, namely, 1430, 1391, 1357, 1015, 958, and 804 cm^{-1} , are absent in the “amorphous” spectrum. In addition, the intensities of these bands were enhanced during crystallization. There-

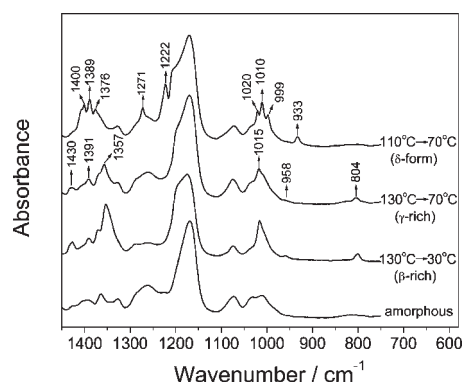


Figure 2. FT-IR spectra in the 750–1450 cm^{-1} region of melt-crystallized PHPs and that of amorphous PHP.

fore, these bands may be related to the arrangements of chain conformation required for the crystallization, and are referred to as “crystalline” bands. The similarity in the shape and location of the “crystalline” bands indicates that the crystalline chains adopt a similar conformation (*trans*^[5–8]) in the β and the γ form. It is also found that CH_2 bending absorptions at 1430 cm^{-1} and CH_2 rocking absorptions at 804 cm^{-1} are doublets rather than singlets, which is possibly attributable to interactions between adjacent crystalline molecules, as reported for polyethylene.^[11] The intensity ratios of the doublet of the β form differ from those of the γ form, possibly indicating their difference in interaction strength.

Similarly, nine “crystalline” bands, that is, 1400, 1389, 1376, 1271, 1222, 1020, 1010, 999, and 933 cm^{-1} are observed in the spectrum of the δ form. All these bands are completely absent in both spectra of the γ and the β form, suggesting that the chain conformation in the δ form is different from the *trans* conformation. Conversely, an absence of the “crystalline” bands of the γ or β form, 1430, 1357, 1015, and 804 cm^{-1} , in the spectra of the δ form again confirms the conformation rearrangement in the δ form.

Conformational Analysis

A previous conformational analysis^[10] predicted that PHP and the α - or β -substituted PHPs would all adopt either the 2_1 helix or the *trans* conformation in the crystalline state. The prediction has been well validated by research on PHP,^[5–8] PHB,^[4,12] polypivalolactone (PPL),^[13] poly(α -methyl- α -*n*-propyl- β -propiolactone) (PMPPL),^[14,15] and poly(α -methyl- α -ethyl- β -propiolactone) (PMEPL),^[15,16] where the crystalline chains take either the 2_1 helix or the *trans* conformation.

As described above, the chain conformation in the δ form differs from the *trans* conformation in the β and the γ form. On the basis of the conformational analysis, it seems reasonable that the polymer chains adopt the 2_1 helix in the δ form of PHP, as also supported by FT-IR results. Isotactic PHBs, adopting the 2_1 helix in the crystalline phase, are isotactic β -methyl-PHPs in terms of the chemical structure. The two bands at 1279 and 1228 cm^{-1} , possibly originating from the C–O stretching mode or CH_2 bending mode, are found to characterize the helix conformation of PHB.^[17] Hence, the presence of the bands at 1271 and 1222 cm^{-1} in the spectra of the δ form seems to indicate the adoption of a 2_1 helix conformation in the δ form, as shown in Figure 2.

Spherulite Growth and Morphology

Under microscopic observation at 70 °C, two populations of spherulites, that is, Type I, with a smooth texture and bright birefringence, and Type II, with a coarse texture and faint birefringence, are found to be formed in PHPs melted at

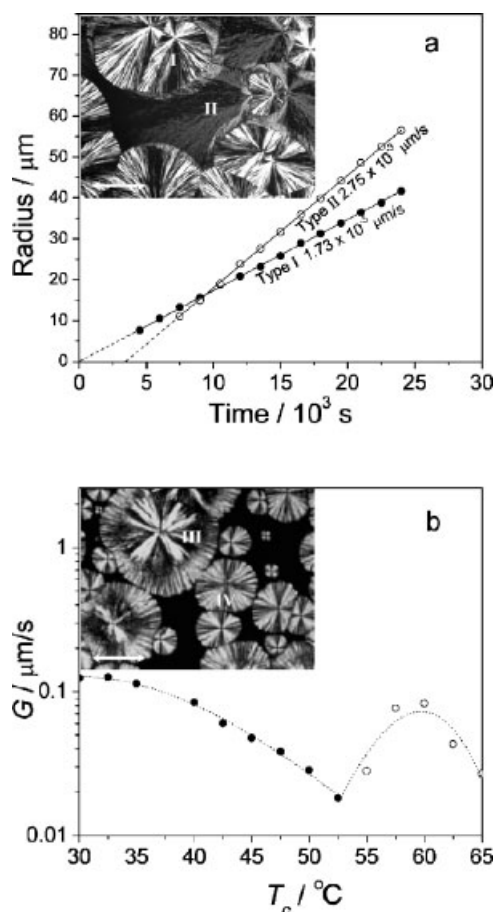


Figure 3. a) The time dependence of the radius for the Type I (δ form) and Type II (γ form) spherulite in PHP quenched from 117 to 70°C ; b) The plot of radial growth rate as a function of T_c for the spherulites in PHPs quenched from 130°C . The dual morphology developed at 70°C after melting at 117°C , and that developed during cooling at $1^\circ\text{C} \cdot \text{min}^{-1}$ from 130°C , are inserted in Figure 3a and 3b, respectively.

110°C and those at 130°C , respectively. When T_f is 117°C , it is of interest to observe that the growth of Type I morphologies is coupled with that of Type II morphologies, as shown in the inserted picture of Figure 3a. With increasing T_f , Type I is nucleated more sporadically, and finally Type I completely disappears in PHPs melted at 130°C . Note that the T_f dependence of spherulite morphology corresponds well to that of the polymorphism. As expected, by FT-IR spectroscopy, Type I and Type II morphologies are distinguishable in the crystalline structure, being the δ form and the γ -rich form, respectively. Reasonably, the alteration of crystalline structures will lead to that of growth kinetics. As shown in Figure 3a, Type II and I grow at a rate of 2.75×10^{-3} and $1.73 \times 10^{-3} \mu\text{m} \cdot \text{s}^{-1}$, respectively, with a difference of a factor of almost 1.59 which finally results in small, tear-shaped Type I spherulites enclosed within large Type II spherulites.

In addition, the microscopy results can explain the T_f -dependent polymorphism. The nucleation density of Type I

is found to decrease with increasing T_f , which seems to correlate with the trend of residual thermal history. Furthermore, compared with the spontaneous nucleation of Type II, the fixed amount of Type I during the crystallization process suggests that its crystallization is nucleated only by some residual nuclei sensitive to T_f . Possibly, these specific nuclei are crystal fragments or short-range intramolecular orders undestroyed during melting of the cast δ form. However, it is still amazing to note that these residual orders can withstand such a high temperature ($<130^\circ\text{C}$), which is about 50°C higher than the melting point of the cast PHPs.

For PHPs melted at 130°C , spherulite growth rates, G_s , measured during the isothermal crystallization were plotted against T_c in Figure 3b. The G value shows an unusual bimodal-shape dependence on T_c . In detail, with undercooling, the G value passes through two maxima with a minimum value of G at 52.5°C . In addition, some distinct morphology differences can be observed between the textures formed at above and below 52.5°C . Spherulites with a rough texture and a distorted Maltese Cross (Type III) formed at above 52.5°C , while another population of spherulites with a smooth texture and a pronounced Maltese Cross (Type IV) nucleate at below 52.5°C . Namely, the spherulite will adopt a different texture when it follows a different growth mode. The distinctive difference in the texture between the two spherulites can be observed clearly in PHPs crystallized during cooling at a rate of $1^\circ\text{C} \cdot \text{min}^{-1}$, as shown in the inserted picture of Figure 3a. The bimodal-shape growing behavior of the spherulites should be related to the T_c -dependent polymorphism. That is, the thermally accessible γ form and the kinetically favorable β -form nucleate more profusely at high temperature and low temperature, respectively, resulting in the bimodal growth dependence of PHP spherulites on T_c .

Conclusion

Depending on the MW, T_f , and T_c , the PHP chains are found to take on a β , γ , and a new δ form. The PHP chains adopt similar *trans* conformations in the β and the γ form, but possibly adopt a 2_1 helix in the δ form. A low MW and a low T_f favor the formation of the δ form; a high MW, high T_f , and high T_c favor the formation of the γ form; and a low T_c favors that of the β form. The growth of the δ form is initialized by some residual nuclei that survive from melting. The low T_f of 117°C allows the δ -form spherulite to nucleate and grow together with the γ -form spherulite, but it follows different kinetics from that of γ -form spherulite. A high T_f of 130°C completely erases any δ -sensitive nuclei, so that only spherulites consisting of the γ form and the β form are present in the PHP, wherein the thermally accessible γ form and the kinetically favorable β form nucleate more profusely at a higher and lower T_c , respectively, resulting in the bimodal spherulite growth behavior.

Interestingly, our most recent examinations indicate that the low-molecular-weight δ -form PHP films have much better mechanical properties and slower enzymatic degradation rate than the β - and γ -form films, even with a much higher molecular weight.^[18] The good mechanical properties and the slow biodegradation of the δ -form PHP are possibly related to the numerous nucleations during crystallization and the adoption of the 2_1 helix conformation, respectively. At present, further investigation is in progress in order to clarify the origin and the crystalline structure of the δ form.

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