Feature Article

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Study of C-H···O Hydrogen Bond for Biodegradable Polymer using Infrared Spectroscopy and X-ray Diffraction

— Role of the “Weak Hydrogen Bond” toward Crystal Structure Stabilization and Thermal Behavior —

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This study focuses attention on the typical biodegradable polymer, PHB (poly (3-hydroxybutyrate)) using Infrared (IR) Spectroscopy and X-ray Diffraction in combination. The study shows research into the crystalline structure and thermal behavior of PHB, culminating in confirmation of C-H···O=C interaction in the PHB crystalline structure for the first time. The C-H···O=C interaction is one of the famous “Weak Hydrogen Bond” situations that have gained much attention recently. We studied the variation of intensity and temperature by considering the slight shift and intensity change between the C-H stretching vibration band and C=O stretching vibration band. For the band analysis, second order derivative and differential spectra were fully applied. Results of quantum chemistry calculation were also taken into consideration. From the stretching vibration band wavenumbers of C-H and C=O, we have investigated the intensity of C-H···O=C hydrogen bond, and researched how the hydrogen bond started breaking along with the temperature rise brought about by intensity change. The C-H···O=C hydrogen bond is said to stabilize the crystal structure by combining two polymer chains each of which has a helical structure. We confirmed the crucial role of C-H···O=C interaction toward the unique “folded” structure of the PHB molecule chain, the crystalline structure stabilization, and the high copolymer crystallization.

Introduction

Plastics mass-produced from fossil fuels are indispensable material popularly and widely used in our daily lives. Yet most of these plastics do not decompose in the natural environment and the rejected material causes serious environmental pollution. Because of its low environmental load, biodegradable polymers are a good solution to resource depletion and waste problems, furthermore to the CO_2 problem that is a main factor of global warming. Studies of biodegradable polymers are being actively pursued inside and outside Japan[1]-[4], yet unfortunately they focus rather on applications such as synthesis and biodegradation, and not on the fundamental physical properties. This is why our team decided to research fundamental properties, covering the crystalline structure and thermal behavior of the biodegradable polymer, poly(3-hydroxybutyrate) (PHB).

Biodegradable Polymer

Biodegradable polymers are mainly classified into three categories; polymers generated by microorganisms, natural polymers found in plants, and synthetic polymers created by chemical synthesis typically represented by polylactic acid. PHB, the typical biodegradable polymer generated by microorganisms (Figure 1) is notable due to its unique crystal growth in which the molecule chains are folded in the direction of the *a*-axis in the crystal lattice[5],[6]. We tried to clarify this unique growth mechanism using IR spectroscopy and X-ray diffraction

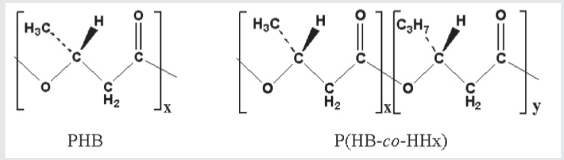

Figure 1 Structure formula of PHB (poly (3-hydroxybutyrate)) and Copolymer

to fully study the crystalline structure and thermal behavior of PHB. In the course of our studies, we found the existence of the C-H···O=C hydrogen bond in the PHB crystalline structure. Recently, the C-H···O=C hydrogen bond has been found in proteins and nucleic acids, gaining attention because of its crucial functions. Now, we will explain more about the C-H···O=C hydrogen bond.

C-H···O=C Hydrogen Bond

Recently there has been much interest in C-H···O hydrogen bonding, it has been recognized as having a very important role in stabilizing the mutual association between proteins and nucleic acids. It stabilizes interactions between enzymes and substrates, stabilizes biomolecule transition states, and of course it stabilizes the individual structures of proteins and nucleic acids. For example, base pairs such as C-G (cytosine-guanine) and U-A (uracil-adenine) are stabilized by three hydrogen bonds, not only by the normal NH···O hydrogen bond but also including the C-H···O=C hydrogen bond. Thorough analyses have been presented on many proteins, to gradually clarify the presence of the C-H···O hydrogen bond common in proteins.

One of the most important features of this study is the new finding that the C-H···O=C hydrogen bond exists in polymers other than biomolecules, and the clarification of the thermal behavior of the lattice constant along with the intensity change of the C-H···O=C hydrogen bonding. This has been achieved by combined use of IR spectroscopy and X-ray diffraction.

Crystal Structure of Biodegradable Polymer and C-H···O=C Hydrogen Bond

Figure 2(a) shows the crystal structure of PHB[7],[8]. The crystal system of PHB is orthorhombic, and the lattice spacing is: a=5.76 Å, b=13.20 Å, c=5.96 Å (fiber axis), with a crystal structure of double helical symmetry for every crystal axis in the space group of $P2_1\ 2_1\ 2_1\text{-}D_2^{\,4}$. Furthermore, from X-ray diffraction measurements, it was apparent that the copolymer P(HBX-*co*-HHx) with hydroxyhexanoic acid (HHx)(Figure 1) also has the same orthorhombic system[9]-[12]. Here we should notice that the oxygen atom of the C=O group and the CH_3 group is aligned helically outward and accordingly the distance between them is considerably small (Figure 2). When the PHB crystal structure was analyzed in 1973, no one noticed the proximity of CH_3 group and the oxygen atom of the C=O group, nor the hydrogen bond of C-H···O=C. So I was in fact the first to draw attention to this[9]-[18]. Notably, the C=O oxygen atom is not equidistant from each of the three CH_3 groups, but have a rather unusual

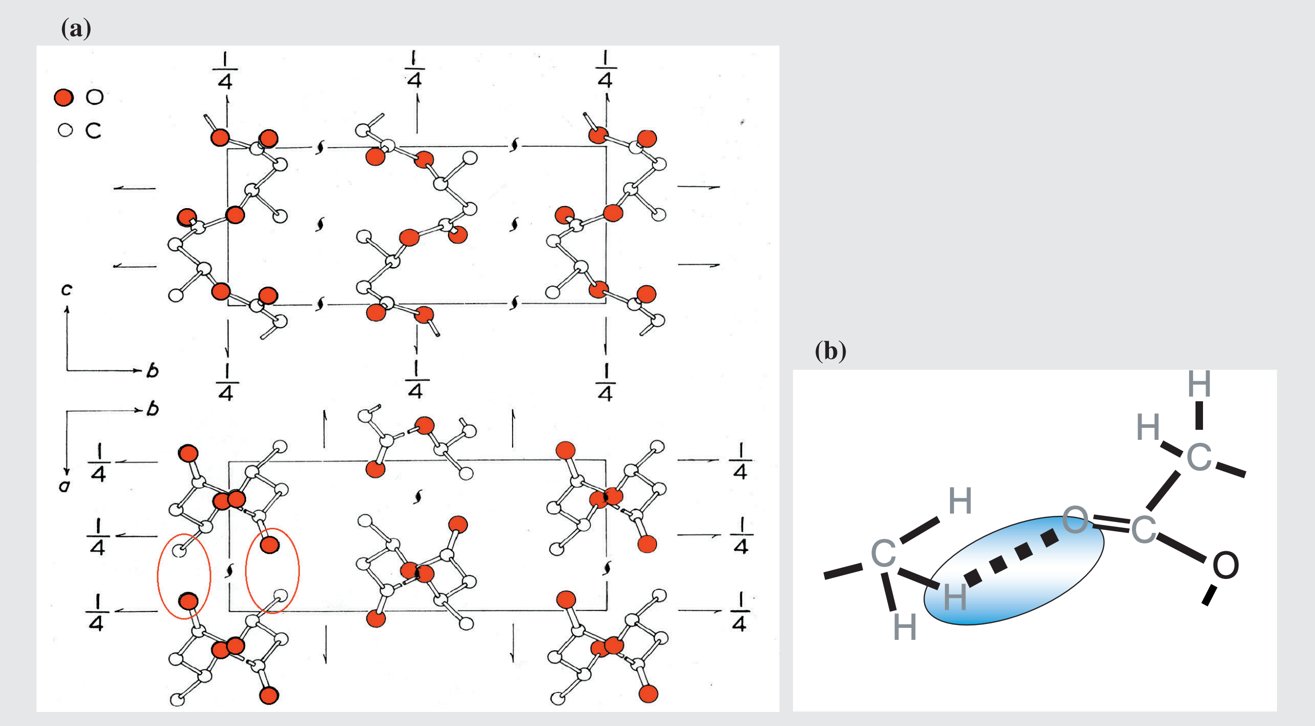

Figure 2 PHB Crystal Structure
(a)Location of Elements (b)Pattern Diagram of C-H···O=C Hydrogen Bond
ʃ : Helical axis perpendicular to paper surface
← : Helical axis parallel to paper surface (Helical axis is positioned at: heights 0 and 1/2 of unit grating for those without numbers, heights 1/4 and 3/4 for those stated as “1/4” in the figure.)

proximity (2.63 Å) with just one of the groups. This distance is even smaller than 2.72 Å, the sum of the Van der Waals radii of oxygen and hydrogen atoms (because the X-ray crystal analysis cannot specify the H atom position, the smallest distance between C-H···O is obtained by rotating the CH$_3$ group with the condition that the C-H bond distance is 1.09 Å.) This implies the existence of weak interaction (C-H···O=C hydrogen bonding) between the oxygen atom and the hydrogen atom. We measured the temperature shift of PHB using X-ray diffraction, and revealed that the temperature shift related change in lattice constant *a* is larger than that in *b*. We also found that the phase interval relating to the lattice constant a gradually extends along with the temperature rise from room temperature. In consideration of the temperature observation and the crystal structure system (Figure 2 (a)), we concluded that a weak interaction (C-H···O=C hydrogen bond, Figure 2(b)) exists between the C=O group and CH$_3$ group, each of which is adjacent within the *a*-axis, and which is further weakened along with the temperature rise to extend the phase interval in the direction of the *a*-axis.

Progress of IR Spectroscopy and Spectral Analysis

Infrared spectroscopy allows direct study of the C-H···O=C hydrogen bond from the bands of C-H stretching vibration and C=O stretching vibration. More specifically, from the wavenumbers the intensity of the C-H···O=C hydrogen bond can be researched, and from the temperature shift generated by the intensity, the status of the gradual bond breakdown can be closely examined (Figure 3). The shift that occurs is so extremely slight that conventional infrared spectroscopy cannot detect it. Thanks to recent advances in FT-IR technology in which the second order derivative and differential spectra have been fully applied, spectral analysis of ultra-high precision is now possible, and this slight amount of shift can be discussed. Figure 3 (a, b) shows the temperature shift of the C-H stretching vibration field (3050-2850 cm^{-1}) for the PHB IR spectra and the second order derivative spectra (3050-2950 cm^{-1}). As can be clearly seen from the comparison of (a) and (b) in Figure 3, the band appears only as a slight "shoulder" in the original spectra (a). (b) clearly shows the peak in the lower direction in the second order derivative spectra, and gives us the clear status of gradual shift to a lower wavenumber along with the temperature shift. Also from the quantum chemistry calculation, the shift in C-H, C=O stretching vibration bands was confirmed. One of the outstanding and important points about this research into the "weak hydrogen bond" was the application of cutting-edge FT-IR technology. The studies also clarified the slight difference between C-H···O=C hydrogen bonds in copolymers and polymer blends from PHB homopolymer through the course of in-depth analysis of the IR spectra. From the temperature shift of the IR spectrum, a breaking and regeneration process of C-H···O=C hydrogen bonds within the molecular chains was clearly evident. There have been no reports of this phenomenon being observed before, so we regard this as a first.

CH$_3$ stretching vibration degeneration is normally monitored in the field ranging from 2980 to 2960 cm^{-1}. PHB shows at least five bands in the field ranging from 3010 to 2960 cm^{-1} (Figure 3). Among the five bands,

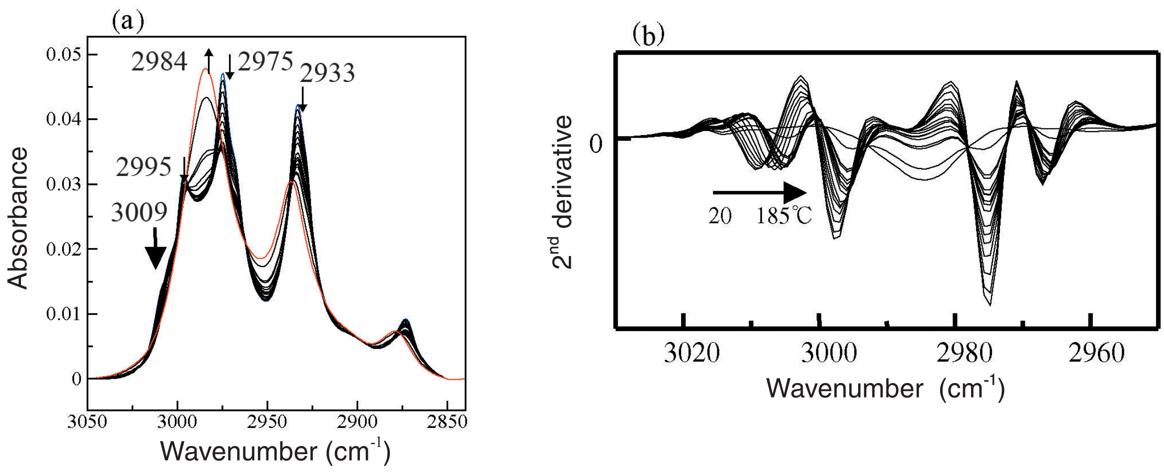

Figure 3 C-H Stretching Vibration Field of PHB Infrared Spectra (a) Temperature Shift (20 to 185 °C) (b) Second Order Derivation

those in fields of 3009, 2996, 2974, and 2967 cm^{-1} belong to a group depending on the crystal structure (whose intensity decreases along with temperature rise), whereas the band in the field of 2984 cm^{-1} belongs to a group depending on the amorphous state (whose intensity increases along with temperature rise). Here we notice the wavenumber for the band in the field of 3009 cm^{-1}. This is a considerably high wavenumber compared with the normal CH_3 degeneration stretching vibration. This is what reveals the existence of the C-H···O hydrogen bond.

On the other hand, the C=O stretching vibration field shows the PHB band depending on the crystal structure at 1723 cm^{-1} (which decreases in intensity along with temperature rise) with the band depending on amorphous state at 1740 cm^{-1} and over (which increases intensity along with temperature rise). Normally, the C=O group of an ester system shows the C=O stretching vibration at around 1750 cm^{-1} and over, the C=O band, depending on the PHB crystal structure, is supposed to shift to a very low wavenumber. This is also evidence of the C-H···O=C hydrogen bond.

We went on to the quantum chemistry calculations on the PHB model compound ($CH_3CH(CH_3)O(C{=}O)CH_3$) to clarify the relationship between the C-H···O=C hydrogen bond and the vibration spectra, and obtained the structure showing the best stabilization and stretching vibrations for C-H and C=O. Figure 4 shows one of the structures showing the best stabilization. In this structure, two C-H ···O hydrogen bonds are formed between two molecules. This structure resembles that obtained from PHB with the X-ray crystal analysis. The distance between the C-H group and the C=O group is 2.56 Å, and the energy for the structure stabilization is 1.22 kcal/mol. The wavenumber shift of the C=O stretching vibration caused by dimerization was 17 cm^{-1} at a lower wavenumber, which was very close to the actual measurement (under1740 to 1723=17cm^{-1}).

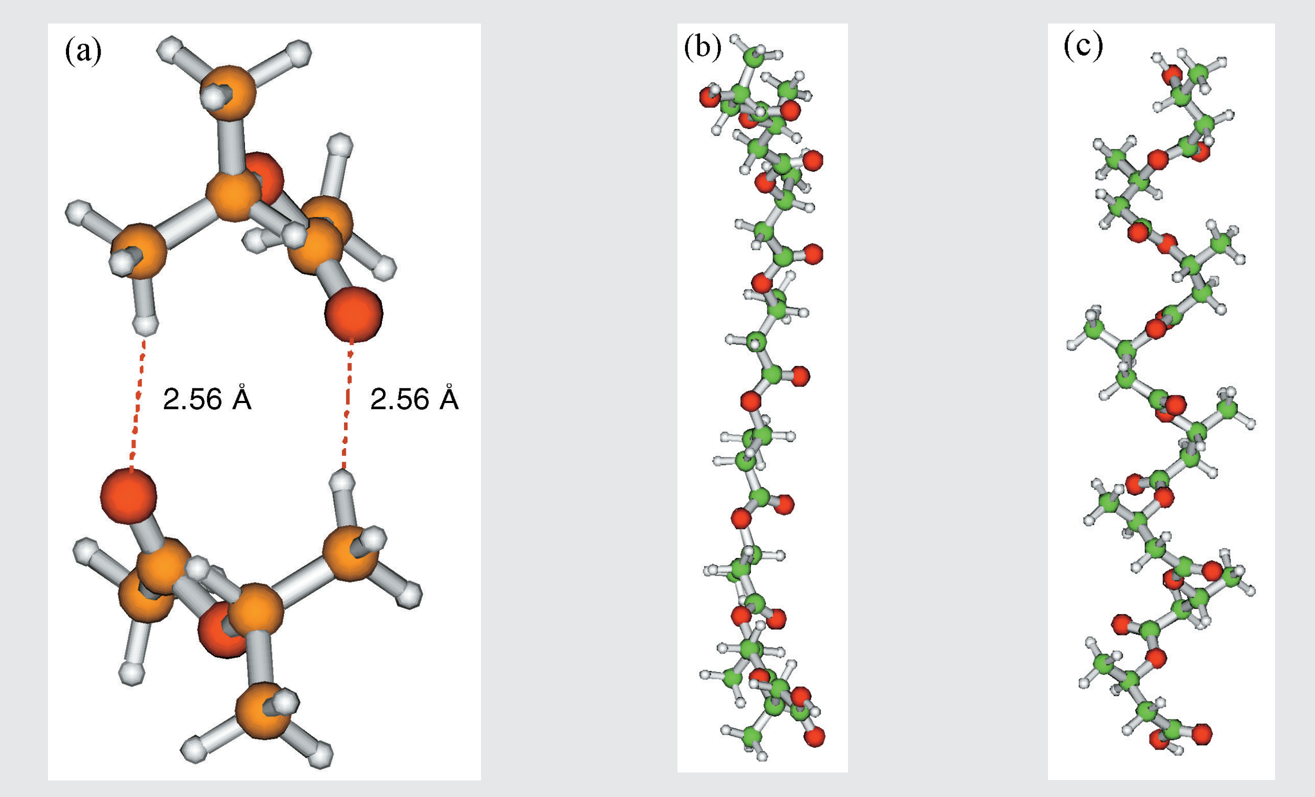

Figure 4 Best Stabilization Structure and Distance(2.564Å)between C-H···Os Estimated by Quantum Chemistry Calculation (B3LYP/6-31+G(d, p))
(a) Two Molecules of Model Compound $CH_3CH(CH_3)O(C{=}O)CH_3$
(b) Model of PHB Phase Zigzag Structure
(c) Model of PHB Helical Structure

Establishing Model Structure

We integrated our study results obtained from infrared spectroscopy, quantum chemical calculation, and X-ray diffraction into a lamella model of a PHB crystal as shown in Figure 5 (a). In the crystals of PHB and its copolymer, their structures show pairs of C-H···O=C hydrogen bonds between each helical structure as shown in Figure 5, which can stabilize the crystal structure.

From measurements of X-ray low angle scattering, the thickness of PHB lamella is known to be approximately 5.2 nm[19]. We believe the existence of the C-H···O=C hydrogen bond to be aligned with the *a*-axis between molecule chains as shown in Figure 5. Lamella thickness suggests the existence of seven to eight pairs of C-H···O=C hydrogen bonds. In the PHB copolymer, the lamella thickness decreases by almost half and the amorphous thickness increases instead. The reason why the random copolymer including 10% or more of HHx keeps crystallization level to some extent, even with a very thin lamella, is considered to be because of the C-H···O=C hydrogen bond present in the helix.

PHB crystal formation starts from flake lamella generated in a molecule chain growing into the *a*-axis, and completes with the self-assembly function. We deduced the reason why the PHB crystal formation grows in the direction of the *a*-axis is because the C-H···O=C hydrogen bond exists in the direction of this axis.

Here let us compare the PHB crystal structure with two typical polymer crystals; polyethylene (PE) and Nylon. PE, a typical polymer for general use is the polymer with the simplest structure. The molecule chains are folded into flake monocrystals (called "lamella"), and multiple lamellas are additionally twisted and folded, so as to form a spherical structure (called a "spherocrystal"). In PE monocrystals separated out from diluted solution, the folding goes in the direction of (110) phase. Whereas PHB is reported to grow by alternatively folding the molecule chains in the reverse direction between the phases (110) and $(1\bar{1}0)$ along the *a*-axis. However, it is unknown why the folding goes along the *a*-axis in the PHB case. We assume

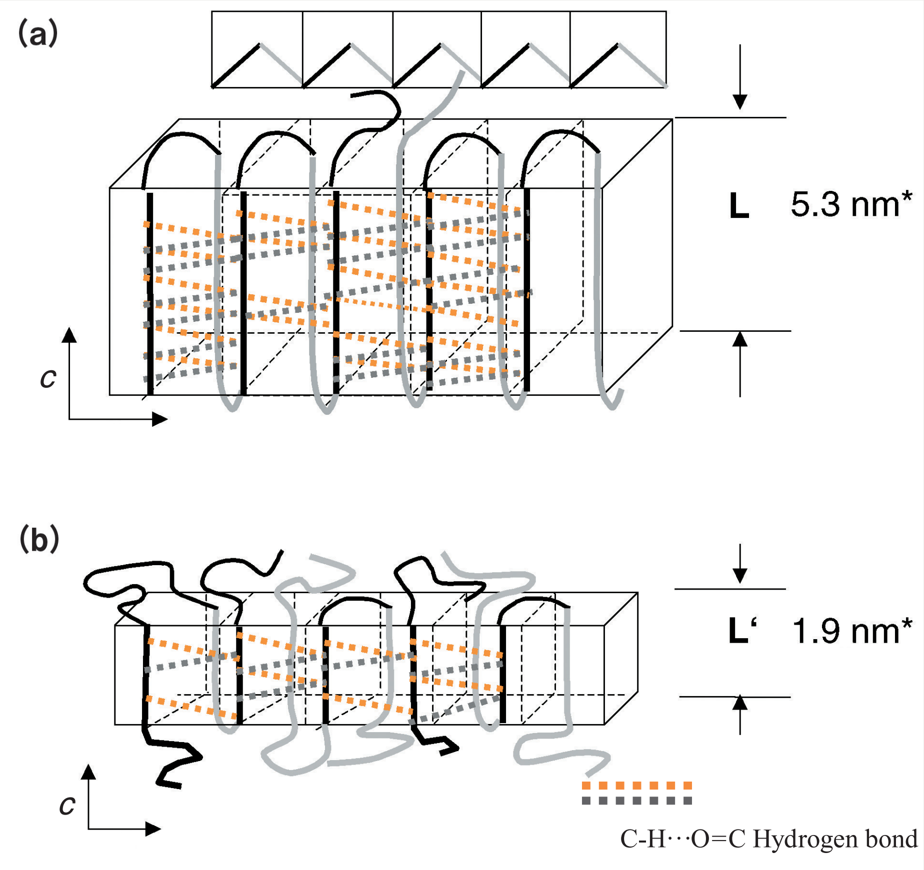

Figure 5 Crystal Structure Models Proposed according to IR Spectra Results (a) PHB (poly (3-hydroxybutyrate)) (b) Copolymer
C-H···O=C hydrogen bond connects helical to stabilize the crystal structure.
* : Reference[19]

the reason is connected with existence of the C-H···O=C hydrogen bond in the PHB crystal structure. On the other hand, Nylon is popularly known for its high strength and malleability, excellent elasticity, and high melting point because of its structure, where an amide group in the main chain forms NH···O hydrogen bonds in molecular chains. For example, the crystalline structure of Nylon 66 has NH···O hydrogen bonds in the direction of the *a*-axis, stabilizing the entire crystal structure. Compared to C-H···O=C hydrogen bonds, NH···O hydrogen bonds have more than four times stronger bonding energy, and in these the lattice spacing does not increase even with temperature rise. Yet in the "weak hydrogen bond" such as PHB (C-H···O=C hydrogen bond), the bond breaks down with rising temperature, causing an increase in lattice intervals.

Conclusion

By combining the use of IR spectroscopy and X-ray diffraction we have pursued our study of the C-H···O hydrogen bond and its function in biodegradable polymer crystal structures. From now, not only studies of C-H··O bonding be greatly advanced but so will the general understanding of the "weak hydrogen bond." The intention is to further pursue studies of the C-H···O hydrogen bond's role in the PHB crystal structure, and even in polymers other than PHB to find the existence of weak interactions such as C-H···O hydrogen bond and its role.

Knowing the unique crystal growth mechanism of PHB will contribute to its optimum application to future product development, because it influences physical properties such as crystallization level or crystallization speed, and restrains secondary crystallization growth which is a huge hurdle of production degradation. By studying and clarifying how the C-H···O hydrogen bond works in a biodegradable polymer crystal structure, a new concept in polymer crystal structures can be achieved. Last but not least, IR spectroscopy has been the leading player in the study.

(Publication members have responsibility for the translation)

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