# Stereoselectivity in the Formation of Crystalline Inclusion Complexes of Poly(3-hydroxybutyrate)s with Cyclodextrins

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## Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides, consisting of 6, 7, or 8 glucose units, and are named α-, *β*-, and *γ*-cyclodextrin.[1] Supramolecular, crystalline inclusion complexes (ICs) organized by noncovalent interactions can be formed by threading CD molecules onto polymer chains. Since the discovery[2] by Harada et al. that α-CD may form a crystalline IC with poly(ethylene glycol) in aqueous solution, many kinds of linear polymeric guests with either hydrophilic or hydrophobic natures were found to have the ability to form ICs with different types of CDs. For instance, an IC of α-CD with the biodegradable poly(*ϵ*-lysine) was obtained[3] recently by Yui et al., and according to literature reports, ICs of CDs and aliphatic polyesters have been studied by several groups.[4−6]

The driving force for the threading process is due to intermolecular hydrogen bonding between neighboring CDs as well as steric compatibility and hydrophobic interactions between host and guest molecules.[7,8] Although the depth of the cavity of these three cyclodextrin molecules is the same (7.9 Å), the diameters of α-, *β*-, and *γ*-CD cavities are ∼4.5, 7, and 8.5−9 Å, respectively, which are very different.[9] CDs have been found to behave selectively when forming crystalline ICs with specific polymer chains. For example, Harada et al. reported[10] that oligoethylene forms an IC with α-CD, although it does not form an IC with *β*- or *γ*-CD. On the contrary, poly(propylene glycol) (PPG) of any molecular weight does not form ICs with α-CD due to the steric hindrance of the side methyl groups, while it forms ICs with both *β*- and *γ*-CD in high yield. Therefore, the cross-sectional area of polymers correlates with the cavity size of the CD with which it forms an inclusion complex.

Two different kinds of poly(3-hydroxybutyrate)s (PHB)s have attracted attention lately, because of their potential pharmaceutical, biomedical, and environmental applications. The first PHB is the optically active poly((*R*)-3-hydroxybutyrate) (i-PHB), which has an isotactic stereosequence and is synthesized and accumulated by a variety of bacteria as a reserve energy source.[11,12] As an alternative to bacterial fermentation, chemosynthesis, through anionic ring-opening polymerization of *β*-butyrolactone, can also produce PHB.[13] However, the product is usually atactic poly((*R*,*S*)-3-hydroxybutyrate) (a-PHB), which is completely amorphous. Because of their different stereosequences, i-PHB and a-PHB may have different cross-sectional sizes in their extended conformations. Therefore, it is anticipated that they will show different behaviors in their formation of ICs with CDs.

In this note, we report that i-PHB and a-PHB may selectively form crystalline ICs with different CDs. To our knowledge, this is the first experimental report that demonstrates IC formation between polymers and CDs is governed not only by their chemical structures but also by their stereochemistries.

## Experimental Section

**Materials.** Cyclodextrins (α-, *β*-, and *γ*-CD) were obtained from Cerestar Co. i-PHB ($M_n = 2.72 \times 10^5$, $M_w/M_n = 2.19$) (from Aldrich) was purified by precipitation into ethanol from 1,2-dichloroethane. a-PHB ($M_n = 1.59 \times 10^4$, $M_w/M_n = 1.08$) was synthesized via anionic ring-opening polymerization of *β*-butyrolactone in bulk with a potassium methoxide/18-crown-6 ether complex (molar ratio = 1:1) as an initiator, according to previous reports.[13,14]

**Preparation of Samples.** To prepare the ICs with a-PHB, *γ*-CD (16 g as received) was dissolved in distilled water (60 mL). a-PHB (0.8 g) was dissolved in acetone (150 mL). Then the polymer solution was added dropwise to the *γ*-CD solution at 60 °C. After stirring for 3 h at 60 °C, the solution was allowed to cool to room temperature while continuously stirring overnight. A white powder was collected by filtration and then washed with acetone and water to remove free polymer and uncomplexed *γ*-CD, respectively. A similar method was attempted in the formation of ICs with α- or *β*-CD. The ICs with i-PHB were prepared by a similar method, except that i-PHB (0.5 g) was dissolved in chloroform (150 mL) and α-CD (8 g) was dissolved in DMSO (18 mL).

**Measurements.** The FTIR spectral studies were carried out with a Nicolet 510P FTIR spectrometer using a resolution of 2 $cm^{-1}$. Solution $^1H$ NMR spectra were recorded on a Bruker 300 MHz DPX spectrometer in DMSO-$d_6$ at room temperature. Solid-state $^{13}C$ NMR data were collected using a Bruker DSX wide-bore system with MAS speeds of 4−5 kHz and CP contact time of 1 ms. DSC measurements were performed at a heating rate of 10 °C/min on a Perkin-Elmer differential scanning calorimeter (DSC-7) calibrated with indium. The thermal decomposition behaviors of samples were measured with a Perkin-Elmer Pyris 1 thermogravimetric analyzer (TGA) at a heating rate of 20 °C/min. X-ray diffraction analysis of powder samples was conducted with a Siemens type-F X-ray diffractometer (30 kV, 20 mA) using Ni-filtered Cu Kα radiation.

## Results and Discussion

When α- or *β*-CD was used to make ICs with a-PHB, all precipitates were readily washed away with acetone and cold water, and no IC product could be obtained. A white powder was collected in high yield in the case where *γ*-CD and a-PHB were used to make an IC. Since i-PHB is insoluble in acetone, we selected chloroform to dissolve i-PHB and to make ICs with CDs. We first tried to make ICs by adding the i-PHB chloroform solution to the CD aqueous solutions. After washing the precipitate with water and chloroform, no IC product was obtained regardless of which CD was used. Dissolution of α-CD in DMSO and then adding the i-PHB/chloroform solution to the α-CD/DMSO solution, or vice versa, resulted in a white powder product that was collected. When *β*- or *γ*-CD was used, no product was obtained after washing the precipitate repeatedly with water and chloroform. The formation of a-PHB-*γ*-CD IC and i-PHB-α-CD IC was proved by FTIR, TGA, $^{13}C$ CP/MAS NMR, and DSC measurements.

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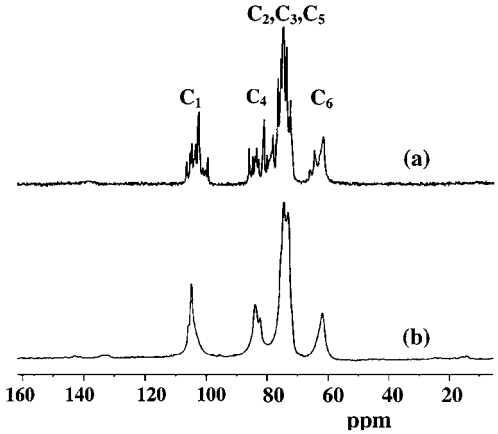


**Figure 1.** $^{13}C$ CP/MAS NMR spectra of $\gamma$-CD (a) and $\gamma$-CD-a-PHB IC (b).

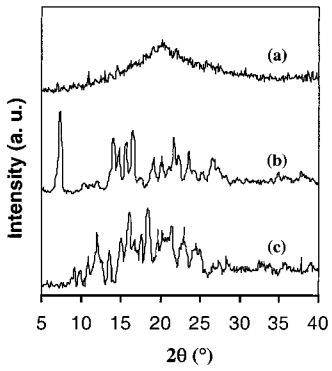


**Figure 2.** X-ray diffraction patterns of a-PHB (a), $\gamma$-CD-a-PHB IC (b), and $\gamma$-CD (c).

FIIR spectra (not shown) of the two ICs indicate the coexistence of CD ($\alpha$- or $\gamma$-CD) and PHB (i- or a-PHB). Because these two products have been sufficiently washed with solvents, i.e., acetone for a-PHB and chloroform for i-PHB, we may reasonably deduce that the PHBs remaining in the powder products have been bound to the CDs in some manner.

The solid-state CP/MAS $^{13}C$ NMR spectra of $\gamma$-CD and $\gamma$-CD-a-PHB IC are shown in Figure 1. The spectrum of $\gamma$-CD in the uncomplexed state shows strong splitting for all $C_{1-6}$ resonances, indicating that $\gamma$-CD molecules are in a rigid, less symmetric cyclic conformation. On the contrary, all $^{13}C$ resonances of $\gamma$-CD in the $\gamma$-CD-a-PHB IC show reduced splitting. This indicates that $\gamma$-CD in the IC has adopted a more symmetric cyclic conformation. Comparable results were obtained for the $\alpha$-CD-i-PHB IC. Similar observations, which are believed to support the formation of ICs between CDs and polymers, have been previously observed in the solid-state CP/MAS $^{13}C$ NMR spectra of crystalline ICs formed between various polymer guests and different CDs.[3,10,15]

WAXD patterns, as shown in Figures 2 and 3, strongly support the formation of $\gamma$-CD-a-PHB IC and $\alpha$-CD-i-PHB IC. In Figure 2, the diffraction pattern of a-PHB only shows a broad diffuse halo, indicating that a-PHB is completely amorphous. The diffraction pattern of the $\gamma$-CD-a-PHB IC is very different from those of $\gamma$-CD and a-PHB. A new strong diffraction peak was observed at 7.6°, which is well-known to be characteristic of $\gamma$-CD-based IC crystals adopting the channel structure.[10,15,16] This peak characteristic of channel structure ICs at 7.6° is not present in the diffraction pattern of pure $\gamma$-CD, which adopts a cage structure.[10,16] In Figure 3, the diffraction patterns of $\alpha$-CD-i-PHB ICs are quite different from those of i-PHB and a-CD. Two prominent diffraction peaks at 13.3° and 16.6° are present in the diffraction pattern of crystalline i-PHB. Major peaks at 9.6°, 12.03°, 19.5°, and 21.8° are observed for pure $\alpha$-CD. For the ICs, two prominent peaks are observed at 20° and 22.6° ($2\theta$), which are well-known to be characteristic of $\alpha$-CD-based IC crystals adopting the channel structure.[3,6,10]

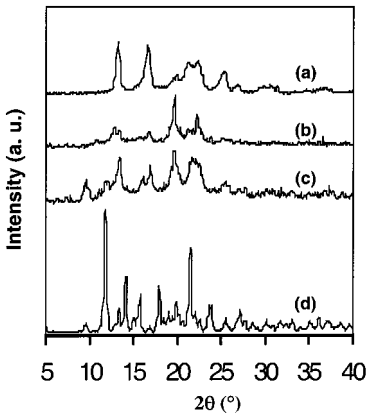


**Figure 3.** X-ray diffraction patterns of i-PHB (a), $\alpha$-CD-i-PHB $IC_1$ prepared by adding i-PHB/chloroform solution to $\alpha$-CD/DMSO solution (b), $\alpha$-CD-i-PHB $IC_2$ prepared by adding $\alpha$-CD/DMSO solution to i-PHB/chloroform solution (c), and $\alpha$-CD (d).

It is noteworthy that the diffraction peaks characteristic of crystalline i-PHB at 13.3° and 16.6° are weak but still recognizable in the diffraction patterns of the $\alpha$-CD-i-PHB ICs. Suspecting that some free i-PHB might have remained in the IC, we washed the IC repeatedly with hot chloroform, which is a good solvent for i-PHB. However, the diffraction patterns of $IC_1$ and $IC_2$ do not show any appreciable change upon washing with chloroform. This result excludes the existence of free i-PHB in the $\alpha$-CD-i-PHB ICs. The only other rational explanation for these results is that i-PHB chains were only partially included, so that the uncomplexed parts of i-PHB chains are still able to aggregate to form a crystalline i-PHB phase coexisting with the IC crystals.

Diffraction peaks from crystalline i-PHB in the diffraction pattern of the IC formed by adding i-PHB/chloroform solution to the $\alpha$-CD/DMSO solution (Figure 3b) are much weaker than that in the diffraction pattern of the IC prepared in the opposite manner (Figure 3c). In addition, the IC prepared in the former case exhibits sharper more intense crystalline IC diffraction peaks at 20° and 22.6°. Therefore, the former method (Figure 3b) favors IC formation between i-PHB and $\alpha$-CD compared with the latter (Figure 3c).

The partial coverage of each i-PHB chain by $\alpha$-CD molecules is also demonstrated by the $^1H$ NMR spectrum of i-PHB-$\alpha$-CD IC in DMSO-$d_6$ (not shown). It is known that the depth of CD molecules is 7.9 Å, which is equal to the length of 1.5 repeat units of PHB. From the integrals of the methine group (C*H*) resonance[13] of i-PHB at ~5.23 ppm and the $C_1H$ resonance[3,9] of CD at ~4.9 ppm, the ratio of i-PHB repeat units to $\alpha$-CD is calculated to be 2.37/1.0 for i-PHB-$\alpha$-CD $IC_1$ (Figure 3b) or 4.26/1.0 for i-PHB-$\alpha$-CD $IC_2$ (Figure 3c), which is much higher than 1.5. In contrast, the ratio of a-PHB repeat units to $\gamma$-CD is calculated to be 1.65, which is close to 1.5. Therefore, each i-PHB-$\gamma$-CD IC channel

should contain nearly fully included a-PHB chains. A possible reason for the partial coverage of i-PHB chains by α-CDs is that the molecular weight of the i-PHB used is too high, and so it may be difficult for the very long i-PHB chains to be fully included by the α-CDs.

The thermal properties of the two PHB ICs were investigated by TGA and DSC measurements (not shown). In the TGA scans, the a-PHB-$\gamma$-CD IC exhibits a single thermal decomposition transition, indicating that no or negligible amounts of free $\gamma$-CD or free a-PHB exist and each a-PHB chain was almost fully included. The thermal decomposition temperature ($T_d \approx 348$ °C) of this product is much higher than the $T_d$ (∼225 °C) of a-PHB and is very close to the $T_d$ (∼342 °C) of $\gamma$-CD. In contrast, due to the partially covered nature of each i-PHB chain, as revealed by WAXD and $^1$H NMR measurements, the i-PHB-α-CD ICs exhibit two thermal decomposition transitions; i.e., a weak decomposition transition of i-PHB is still detectable. For the a-PHB-$\gamma$-CD IC, no fusion or glass transition were observed in the heating run of its DSC thermogram. On the contrary, a very weak fusion peak at ca. 174 °C corresponding to an i-PHB crystalline phase is still detectable in the DSC measurements of α-CD-i-PHB ICs. It is noteworthy that the melting temperature ($T_m$) of the i-PHB crystalline phase in the α-CD-i-PHB ICs is lower than the $T_m$ of pure i-PHB (ca. 185 °C), possibly due to the perturbation of crystallization resulting from the partial inclusion of i-PHB chains.

On the basis of the results and discussion above, it is concluded that a-PHB may form an IC with $\gamma$-CD. However, a-PHB is not able to form ICs with α- or $\beta$-CD. Because of the side methyl groups and their atactic attachment, the cross-sectional area of a-PHB is larger than, e.g., poly($\epsilon$-caprolactone) (PCL) and poly(L-lactide) (PLLA), which have been demonstrated to form ICs with α-CD. Therefore, the α- or $\beta$-CD cavity is likely too small to accommodate the a-PHB chain in low-energy extended conformations necessary for inclusion. In contrast, i-PHB only forms an IC with α-CD, while $\beta$- and $\gamma$-CD did not form crystalline complexes with i-PHB.[17] Their cavity sizes might be too large for the close steric fit with an i-PHB chain that is required for inclusion.

As a part of the investigation[18,19] of the inclusion behavior between guest PLLA and host urea to form a PLLA-U IC, extended conformations of PLLA and its atactic counterpart poly(DL-lactide) (PDLLA) were modeled[18] for the purpose of finding conformations of PLLA and PDLLA that were narrow enough in their cross sections to fit in the ∼5.5 Å hexagonal channels formed by the IC host urea. It was found that the all-trans conformation of isotactic PLLA could be accommodated in the U IC channels, while no sufficiently narrow conformations were found for the atactic PDLLA chain. In fact, it was suggested that the narrowest PDLLA conformations had a cross-sectional diameter of ∼7.5 Å. Because the channel diameters of α-, $\beta$-, and $\gamma$-CD are ∼4.5, 7, and 8.5−9 Å, respectively, and because the chemical and stereochemical structures of PLLA and i-PHB and PDLLA and a-PHB are closely similar, it may not be too surprisng that only α-CD forms an IC with i-PHB, while a-PHB is only complexed by $\gamma$-CD.

The results reported in this note may represent a novel way to separate mixtures of polymers with the same chemical but different stereochemical structures.

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(17) It should be noted that the dimer of i-PHB (i-$PHB_2$) has been reported to form a water-soluble IC with $\beta$-CD, but not with α- or $\gamma$-CDs [Li, J.; Uzawa, J.; Doi, Y. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1953]. In solution the i-$PHB_2$/$\beta$-CD stoichiometry was found to be 1/1, and the i-$PHB_2$ residue terminated with the carboxylic acid, the hydroxyl group was suggested to be partially included in the narrow, wide end of the $\beta$-CD bracelet, based on the magnitudes of the shifts in $^1$H and $^{13}$C NMR resonance frequencies between free and complexed i-$PHB_2$ observed in aqueous solution. A 1/1 i-$PHB_2$/$\beta$-CD stoichiometry is equivalent to a 2/1 PHB repeat unit/$\beta$-CD stoichiometry, likely indicating that not all of each i-$PHB_2$ is fully included in each $\beta$-CD, because, as mentioned earlier, a fully included PHB repeat unit/$\beta$-CD stoichiometry of 1.5/1 is expected.
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