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The domain structure and mobility of semi-crystalline poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate): A solid-state NMR study

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Abstract

Solid-state NMR techniques have been employed to investigate the domain structure and mobility of the bacterial biopolymeric metabolites such as poly(3-hydroxybutyrate) (PHB) and its copolymers poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) containing 2.7 mol% (PHBV2.7) and 6.5 mol% (PHBV6.5) 3-hydroxyvalerate. Both single-pulse excitation with magic-angle spinning (SPEMAS) and cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR results showed that these biopolymers were composed of amorphous and crystalline regions having distinct molecular dynamics. Under magic-angle spinning, ^{1}H $T_{1\rho}$ and ^{13}C T_1 showed two processes for each carbon. Proton relaxation-induced spectral editing (PRISE) techniques allowed the neat separation of the ^{13}C resonances in the crystalline regions from those in the amorphous ones. The proton spin–lattice relaxation time in the tilted rotating frame, H $T^{T}_{1\rho}$, measured using the Lee–Goldburg sequence with frequency modulation (LGFM) as the spin–locking scheme, was also double exponential and significantly longer than ^{1}H $T_{1\rho}$. The difference between H $T^{T}_{1\rho}$ for the amorphous and crystalline domains was greater than that of ^{1}H $T_{1\rho}$. Our results showed that the H $T^{T}_{1\rho}$ differences could be exploited in LGFM–CPMAS experiments to separate the signals from two distinct regions. ^{1}H spin-diffusion results showed that the domain size of the mobile components in PHB, PHBV2.7 and PHBV6.5 were about 13, 24 and 36 nm whereas the ordered domain sizes were smaller than 76, 65 and 55 nm, respectively. The results indicated that the introduction of 3-hydroxyvalerate into PHB led to marked molecular mobility enhancement in the biopolymers.

Keywords: Solid-state NMR; Poly(3-hydroxybutyrate); Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

1. Introduction

Polyhydroxyalkanoates (PHAs) are the biopolymeric metabolites of gram-positive and gram-negative bacteria from more than 70 different genera [1] in the form of polyesters of hydroxyalkanoates (or hydroxylated fatty acids). The biopolymers can be accumulated intracellularly up to 90% of the cell dry weight, acting as a carbon and energy reserve [2,3]. PHAs are normally biosynthesized from carbohydrate (or carbon dioxide and water) and readily degraded into hydroxycarboxylic acids, which are normal endogenous metabolites of living systems; ultimately the degradation products are water and carbon dioxide, producing no new or extra carbon dioxide loads to the environment. Therefore, PHAs are renewable, biodegradable, biocompatible and environmental friendly natural alternatives to the petroleum based materials.

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As a class of optically pure PHAs, PHB has been found widespread [4] in the living systems including the cytoplasmic membrane and cytoplasma of *Escherichia coli*, in the membrane of yeast, plants and animals. Upon biodegradation, it produces 3-hydroxybutyrate (3HB), which is a mammalian endogenous metabolite also known as ''ketone body'' hence enabling PHB to have excellent biocompatibility particularly with the mammalian systems. Such biodegradability [5,6] and biocompatibility [7,8] together with the renewable and environmental friendly nature make PHB particularly attractive for many applications such as in biomedical materials [9], drug delivery systems [10], food packaging and biodegradable plastics [11]. With potential applications as the major driving force, many copolymers of 3HB have also been prepared *via* biosynthesis, such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV). It has been found that the introduction of the medium and long side chain hydroxyl fatty acids results in pronounced changes in the physical properties of PHB especially its elasticity [8].

It is conceivable that the structural, dynamic and interaction properties of PHB and its copolymers are key factors governing their properties in biological functioning and functionality in applications. The elucidation of such properties is, therefore, fundamentally important to understand the mode of actions of these polyesters in the living organisms and the functionality in their applications. Extensive researches have been carried out on PHB and some of its copolymers using a variety of techniques including DSC [12,13], X-ray diffraction (XRD) [14,15] and NMR [16–19]. Among many physical techniques, high-resolution solid-state NMR is not only non-invasive and non-destructive method to facilitate *in situ* investigations but also able to provide information on molecular structure, dynamics and interactions of polymers, especially natural polymers [20–22].

XRD results have shown that PHB and PHBV (Fig. 1) have similar crystalline structures; both have 2_1 helical conformations with the fiber periods of 0.596 and 0.556 nm, respectively [23]. The crystallinity of PHBV was in the range from 62% to 69% depending on the HV concentration and decreased with the increase of 3-hydroxyvalerate (HV) content [24]. DSC results [25] showed that, compared with PHB, PHBV has lower glass transition temperature (T_g), melting point (T_m) and crystallinity. PHBV also showed reduced brittleness and enhanced elasticity [8], which may be related to their different microstructural and molecular dynamic properties.

Solid-state NMR studies revealed that the degree of crystallinity (X_c) is higher in PHB (about 70%) than PBHV and some discrepancy was evident in X_c values obtained from the CPMAS and SPEMAS NMR experiments [15–18,26]. For

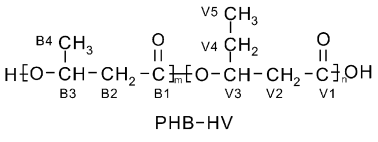

Fig. 1. Schematic representation of the chemical structural features of poly-(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate).

both PHB and PHBV, the X_c values obtained from CPMAS NMR experiments were consistently greater than those from SPEMAS NMR [15–18,26] presumably owing to the ^1H $T_{1\rho}$ relaxation during the contact time in CPMAS NMR even though no authors have discussed about this point so far. The co-crystallization and isomorphism phenomena of PHBV containing more than 30 mol% HV have been extensively studied using high-resolution solid-state ^{13}C NMR spectroscopy and DSC [27–29]. Results showed that two crystalline phases, α-phase and β-orthorhombic phase, existed apart from the amorphous phase. PHBV containing 14–98 mol% HV has also already been studied [28,30] in terms of the co-monomer compositional distribution, thermal and morphological characteristics using WAXD, DSC and solid-state NMR. The results showed that when the HV content was lower than 47 mol%, PHBV has similar crystalline lattice to that of PHB with the HV unit as the crystal constituent whereas when the HV content was higher than 52 mol% crystalline lattice appeared to be similar to that of poly(3-hydroxyvalerate). Furthermore, whilst the T_g value of PHBV decreased consistently with the increase of 3HV content, the T_m and ΔH showed a minimum when 3HV was about 40–50 mol% [28,30]. In contrast, PHBV containing less than 14 mol% 3HV seemed to be less extensively studied. Random copolymers of P(3HB–3HV) with 0–27 mol% 3HV have been examined [31] using ^{13}C CPMAS NMR to determine the crystallinity and the partitioning of the minor fraction residues of 3HV into the crystalline region PHB-type lattice. The signals of the crystalline and those of the amorphous regions were separated according to their different $^1H_{1\rho}$ relaxation [31]. The X_c values were obtained from the amplitude of the two components by spectral deconvolution of the ^{13}C CPMAS NMR spectra. These X_c values are still expected to be overestimated due to ^1H $T_{1\rho}$ relaxation decay even during the short (0.7 ms) contact time.

Less work has been done for the PHBV containing low 3HV monomer (<10 mol%) and the structural features related to the local mobility properties of PHB/PHBV are even less researched though these properties may be important in understanding the physical properties of the biopolymers. Consequently, a number of questions remain unanswered such as 'what are the domain sizes of crystalline and amorphous regions in PHBV', 'how does 3HV content affect the domain sizes', 'what is the nature of the molecular dynamics for these domains', 'are there any relationships between the structural and dynamic properties and their macroscopic properties', if yes, 'what is the relationship'. To answer some of the questions, solid-state NMR is the method of natural choice since it can provide information in molecular structure, dynamics and interactions non-invasively and non-destructively; hence *in situ* information may be obtained. For instance, in multiple domain biopolymer systems, multiple processes are often observed for ^1H $T_{1\rho}$ and T_1 owing to inefficient spin diffusion on these time scales. This provides a unique opportunity to probe the structural and dynamic properties using proton relaxation-induced spectral editing (PRISE) techniques. It has shown as in the examples of plant cell wall systems [32] and the microstructure of native starch granules [33] that