Carbon-13 Nuclear Magnetic Relaxation Measurements of Poly(4-hydroxybutyrate) and Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) in the Bulk

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ABSTRACT: 13C NMR spectroscopy is used to study the local segmental dynamics in the bulk of biodegradable poly(4-hydroxybutyrate) and a random copolymer of 3-hydroxybutyrate and 4-hydroxybutyrate that contained 18% 4-hydroxybutyrate units. The crystalline phase of the copolymer is shown by solid-state 13C NMR to be comprised of 3HB units, in accord with reports based on X-ray diffraction. 13C relaxation measurements indicate that the local dynamics in the amorphous phase are of different time scale for the two different types of monomer units in the copolymer. Furthermore, the segmental motions of the 4-hydroxybutyrate units are found to be slower in the copolymer than in the homopolymer.

Introduction

Poly(hydroxyalkanoate)s, PHA's, are synthesized intracellularly by a variety of bacteria and are used as a carbon and energy reserve.1 As the chemical composition of the polymer can be controlled by the nature of the substrate used for the fermentation, a wide variety of homopolymers and copolymers of the PHA family has been synthesized by this technique.2,3 Most of them are readily biodegradable under various conditions, and possess a wide range of physical properties depending on chemical structure. Although high cost has prevented their widespread use as environmentally-friendly plastics, increasing interest is being expressed for their potential use in drug release systems.4 Poly(4-hydroxybutyrate) (1) and, more recently, copolymers of 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) (2), hereafter referred to as P4HB and P3/4HB, respectively, covering the complete compositional range have been synthesized by fermentation techniques.4 These copolymers are attractive, since the respective homopolymers have quite different physical properties. P3HB is crystalline (50% or greater) and brittle (Tg = 4 °C), while P4HB is a thermal elastomer4 (Tg = −48 °C) of low crystallinity. Several reports on the variation of the physical and mechanical properties of P3/4HB copolymers with composition have been presented.1,4 For instance crystallinity decreases1,4 from 60 to 45% on going from 0 to 16% 4HB content, while at the same time the elongation to break increases from 5 to 444%. By comparison, copolymers of 3HB and 3-hydroxyvalerate (3HV) maintain a high degree of crystallinity at all compositions.1

In this we have used 13C NMR spectroscopy to examine the conformation and dynamics of these systems in the amorphous phase on a repeat-unit level. It has been shown that the 13C NMR relaxation technique is well suited for the study of the segmental dynamics of amorphous6 and semicrystalline8 polymers, and the field has been reviewed recently.9 This paper reports preliminary results of 13C NMR relaxation experiments of the amorphous phase of semicrystalline P4HB and a random copolymer containing 18% (by 1H NMR in 1,1,2,2-tetrachloroethane-d4) 4HB units (P3/4HB-18). Samples for high-resolution spectra were prepared by placing finely ground pieces (~300 mg) of the polymer in 5 mm tubes, heating above the melting point until a homogenous melt phase was produced, and holding them at room temperature for several days prior to conducting any experimental work.8 High-resolution scalar-decoupled spectra (Figure 1a,b) were recorded on a Varian Unity-500 instrument operating at 125.7 MHz for the carbon nucleus. Relaxation experiments were performed on a Varian XL-300 instrument, with a 13C operating frequency of 75.4 MHz. 13C T1 relaxation times were measured by the standard inversion—recovery technique, with delays between acquisitions equal to 5 times the longer T1 measured, using a three-parameter nonlinear fit procedure. NOE's were measured by gated decoupling, with delays between acquisitions equal to 10 times the longer T1. The temperature was controlled to within ±1 °C by standard instrument hardware. No lock solvent was employed. Solid-state NMR spectra (Figure 1c,d) were acquired on a Chemagnetics CMX-300 instrument operating at 75.4 MHz for the 13C nucleus, with a pulse delay of 3 s between acquisitions, spinning rate equal to 3.5 kHz, and high-power proton decoupling of ~60 kHz. For the spectrum in Figure 1d, a contact time of 2 ms was used. Chemical shifts are referenced to TMS, using hexamethylbenzene as a standard (methyl peak at 17.35 ppm). Samples for solid-state NMR were in the form of a powder.

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The $^{13}$C NMR spectrum of P3/4HB-18, under the same experimental conditions as those of P4HB (Figure 1a) is depicted in Figure 1b. Peaks originating from the amorphous 4HB units of the copolymer can be identified in this spectrum. However, they are much broader than the respective peaks in the homopolymer spectrum (Figure 1a). Morin and Marchessault\textsuperscript{11} reported that the amorphous component of a P3HB sample did not show any $^{12}$C spectrum at 68°C under conventional conditions. However, peaks originating from 3HB units in amorphous regions of P3/4HB-18 can be clearly identified in the spectrum of Figure 1b. The 3HB unit chemical shifts are in agreement with those reported for P3HB in native cells by Barnard and Sanders.\textsuperscript{12} Thus, the inclusion of just 18% flexible 4HB units increased backbone mobility in amorphous regions of P3/4HB-18 sufficiently for 3HB units to be “visible” through conventional $^{13}$C NMR techniques. Figure 1c presents the one-Pulse/Bloch decay/MAS $^{13}$C NMR spectrum of the copolymer P3/4HB-18 at room temperature. In such a spectrum, peaks arising from monomer units in amorphous regions of the sample are enhanced with respect to those in crystalline regions. In spectrum 1c broad peaks corresponding to amorphous 3HB and 4HB monomer units can be identified, along with peaks from 3HB crystalline regions. The crystalline and amorphous 3HB peaks are severely overlapping; nevertheless their chemical shifts are in perfect agreement with those reported by Doi et al.\textsuperscript{13} for P3/4HB copolymers of high 4HB content.

Figure 1d presents the CP-MAS spectrum of P3/4HB-18 at room temperature. The absence of any peaks from 4HB units in the spectrum of Figure 1d indicates that in the P3/4HB-18 copolymer, crystalline regions are comprised of 3HB units only. This is in agreement with X-ray diffraction studies of P3/4HB copolymers as a function of their composition.\textsuperscript{5,14} These studies have shown that in copolymers of small 4HB content (0–28%), the crystal lattice is of the P3HB type, whereas copolymers with more than 60% 4HB units have the P4HB type lattice. Moreover, recent molecular mechanics calculations showed that 4HB units cannot be incorporated in the P(3HB) type lattice. It should be emphasized that this is in contrast to the case of copolymers of 3HB/4V, where 3V units are able to cocrystallize in the P3HB lattice and form isomorphic crystals.\textsuperscript{15}

### $^{13}$C NMR Relaxation Parameters

The fact that well-resolved $^{13}$C NMR spectra can be acquired for P4HB and P3/4HB-18 at temperatures well above $T_g$ allows the measurement of the $^{13}$C NMR relaxation parameters of the protonated carbon atoms of these polymers as a function of temperature. The theoretical framework that links the local motions of polymer chains at a repeat-unit level with their $^{13}$C relaxation behavior is well established.\textsuperscript{9} Although originally derived for polymer solutions, this methodology has been successfully applied to amorphous polymers above $T_g$.\textsuperscript{5,8,11,12}

Figure 2 presents the $^{13}$C longitudinal relaxation times $T_1$ of the carbon atom C-3 in P4HB and carbon atoms C-3 and C-5 in P3/4HB-18 as a function of temperature at a Larmor frequency of 75.4 MHz. $N$ is the number of protons directly attached to the carbon of interest, and $N = 2$ holds for all carbon atoms in Figure 2. Note that since we wish to compare data from different polymers, it is important to use exactly the
same structural position on the main chain. As seen in Figure 2, $^{13}$C NT$_1$ relaxation times increase monotonically with increasing temperature for P4HB. Similar curves were obtained for the C-1 and C-2 carbon atoms of P4HB (not shown). Thus, the local dynamics of the P4HB backbone in the temperature range studied fall in the region of fast motion (extreme narrowing region), and the minimum in the NT$_1$ vs 1/T curve should be reached at somewhat lower temperatures. No discontinuity is observed as the melting point ($T_m = 54$ °C) of P4HB is crossed, as expected. It has been shown that the $^{13}$C relaxation times NT$_1$ in the amorphous phase of semicrystalline polymers do not depend on the degree of crystallinity.$^{7,8}$

The mobility of the 4HB units is significantly smaller in P3/4HB-18 as compared to that in the homopolymer P4HB. This is evidenced by the lower NT$_1$ values for the 4HB units in the copolymer relative to P4HB over the whole temperature range. In fact, the 4HB curve in Figure 2 passes through a relatively broad minimum in the temperature range 40-50 °C. This retardation effect on the mobility of the 4HB units in P3/4HB-18 can be attributed to the presence of less flexible 3HB units in the backbone of this 3HB-rich copolymer. Indeed, inspection of Figure 2 reveals that the 3HB units in the copolymer have lower NT$_1$ values than the respective 4HB units, indicating lower mobility. Additionally, the NT$_1$ minimum for the 3HB units is reached at a temperature of approximately 65 °C, about 20 °C higher than that of the 4HB units.

The difference in mobility between 3HB and 4HB units can be attributed to their different structures. In general, side chain polymers have been shown to be less flexible than linear polymers in dilute solution,$^9$ due to steric interactions between side groups and/or the main chain. In the amorphous phase, the effect of interchain interactions becomes important, too, restricting backbone motion even further. Thus, the linear 4HB unit is expected to have more motional freedom than the methyl-bearing 3HB unit, as is observed in this study.

The above qualitative remarks regarding backbone mobility in PHA's drawn from the variation of $^{13}$C NT$_1$ relaxation times with temperature and chemical structure are further supported by nuclear Overhauser enhancement (NOE) measurements, presented in Figure 3. The NOE's of carbon atoms in the backbone of amorphous polymers usually decrease with decreasing temperature, often approaching a field-independent limiting NOE value much larger than that theoretically predicted$^{9,17}$ for very slow segmental motion. At higher temperatures, a larger NOE value can be used as an indication of increased mobility. The data in Figure 3 show some evidence of a low-temperature plateau for the 3HB units in the copolymer. At a given temperature NOE's are seen to decrease in the order P4HB > 4HB > 3HB. This is the same order of mobility suggested by the data of Figure 2, based on the temperature at which NT$_1$ adopts its minimum value for each monomer unit.

It is noted that our results pointing to slower 4HB unit dynamics in the copolymer relative to the homopolymer are in agreement with those of Dekmejian et al.$^{18}$ These authors studied linear polyethylene (PE) and a series of ethylene-vinyl acetate copolymers in the bulk by the $^{13}$C NMR relaxation technique and reported a significant slowing down of PE dynamics with increasing vinyl acetate content above 17.8 mol %.

**Conclusions.** We have shown that $^{13}$C NMR relaxation experiments can be used to give insight to the local chain dynamics of the amorphous component of semicrystalline PHA's. Trends in relaxation parameters can easily be interpreted in terms of local chain dynamics and backbone flexibility. However, it should be stressed that relaxation data alone can produce, at most, only qualitative information on the backbone dynamics of polymers. Quantitative studies of the type, rate, and amplitude of the various local motions taking place in the polymer backbone require the measurement of multifield/variable-temperature relaxation data and their subsequent analysis with suitable dynamic models.$^{9,15}$ $^{13}$C relaxation studies on P3/4HB copolymers of higher 4HB content are also desirable. Finally, a comprehensive study by solid-state (CP/MAS) NMR relaxation techniques,$^1$ capable of probing slower and near-static motions (Hz to kHz region) in both crystalline and amorphous regions would shed further light on the dynamics in the solid state. These experiments are currently in progress.

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References and Notes
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