

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

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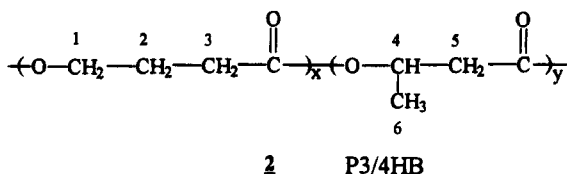
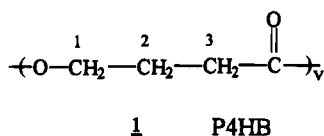
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ABSTRACT: ¹³C NMR spectroscopy is used to study the local segmental dynamics in the bulk of biodegradable poly(4-hydroxybutyrate) and a random copolymer of 3-hydroxybutyrate and 4-hydroxybutyrate that contained 18% 4-hydroxybutyrate units. The crystalline 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.^{1,2} 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.³

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 ($T_g = 4$ °C), while P4HB is a thermal elastomer⁵ ($T_g = -48$ °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 decreases^{1,4} 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 semicrystalline^{7,8} 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-*d*₄) 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 T_1 relaxation times were measured by the standard inversion-recovery technique, with delays between acquisitions equal to 5 times the longer T_1 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 T_1 . 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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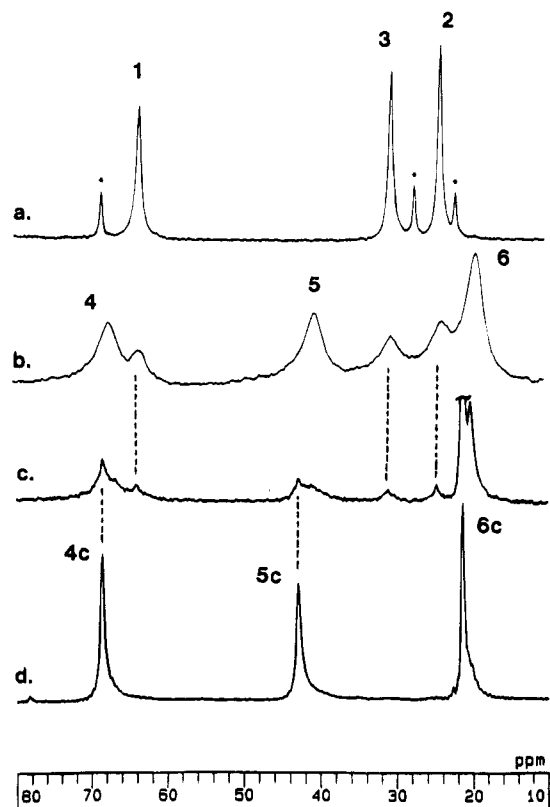


Figure 1. (a) 125.7 MHz ¹³C NMR spectrum of amorphous P4HB at 50 °C. Minor peaks marked with an asterisk are due to γ -butyrolactone (5%) produced by thermal treatment during sample preparation. (b) 125.7 MHz ¹³C NMR spectrum of amorphous P3/4HB-18 at 50 °C. (c) 75.4 MHz one-pulse/Bloch decay spectrum of P3/4HB-18 at room temperature. (d) 75.4 MHz ¹³C CP/MAS NMR spectrum of P3/4HB-18 at room temperature. For numbering see text. Plain numbers denote amorphous peaks, while crystalline ¹³C peaks are marked with a "c".

¹³C NMR Spectra

It is well known⁶ that amorphous polymers well above their glass-transition temperature gave rise to sharp ¹³C NMR spectra by means of conventional high-resolution techniques normally used in the case of liquids. This is also the case for the amorphous component of semicrystalline polymers.^{7,8} Cross polarization (CP) and magic angle spinning (MAS) are not needed in this case, because the rapid and nearly isotropic motion of the polymer chains results in averaging the dipolar interactions and chemical shift anisotropy responsible for line broadening in solids. Figure 1a depicts the ¹³C NMR spectrum of the amorphous component of semicrystalline P4HB (25 ± 5% crystallinity by differential scanning calorimetry).⁵ This spectrum was recorded at 50 °C at a ¹³C Larmor frequency of 125.7 MHz, and only the aliphatic part is shown. At this temperature P4HB is only slightly below its melting point of 54 °C. As seen in Figure 1a, all three methylene backbone carbons of amorphous P4HB give rise to sharp peaks, with line widths in the range 70–80 Hz. Line widths of this magnitude are not unusual for amorphous polymers at temperatures in the vicinity of $T \approx T_g + 100$ °C.^{5,8} The minor peaks denoted with an asterisk in Figure 1a are due to small amounts (~5% by ¹³C NMR) of γ -butyrolactone, produced by slight thermal degradation of P4HB during sample preparation. Abate *et al.* have recently shown that the thermal decomposition of P4HB yields γ -butyrolactone and some higher cyclic oligomers.¹⁰

The ¹³C 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 Marchessault¹¹ reported that the amorphous component of a P3HB sample did not show any ¹³C 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.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 ¹³C NMR techniques. Figure 1c presents the one-pulse/Bloch decay/MAS ¹³C 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.*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.^{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 calculations¹⁵ 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/HV, where 3HV units are able to cocrystallize in the P3HB lattice and form isomorphous crystals.¹⁶

¹³C NMR Relaxation Parameters

The fact that well-resolved ¹³C NMR spectra can be acquired for P4HB and P3/4HB-18 at temperatures well above T_g allows the measurement of the ¹³C 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 ¹³C relaxation behavior is well established.⁹ Although originally derived for polymer solutions, this methodology has been successfully applied to amorphous polymers above T_g .^{6,9,17,18}

Figure 2 presents the ¹³C longitudinal relaxation times NT_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

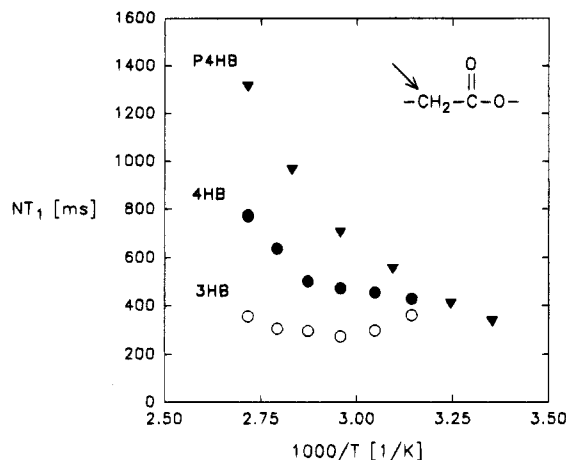


Figure 2. ^{13}C longitudinal relaxation times NT_1 (in ms) of the methylene carbon directly attached to the carbonyl group (atom C-3 in P4HB and atoms C-3 and C-5 in P3/4HB-18; see text) for amorphous P4HB and P3/4HB-18 at 75.4 MHz as a function of temperature.

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),⁹ 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 ($T_m = 54^\circ\text{C}$) 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 $^\circ\text{C}$. 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 $^\circ\text{C}$, about 20 $^\circ\text{C}$ 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

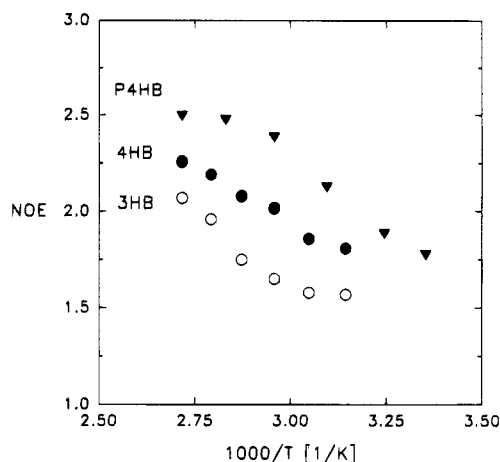


Figure 3. Nuclear Overhauser enhancement (NOE) values of the methylene carbon directly attached to the carbonyl group (atom C-3 in P4HB and atoms C-3 and C-5 in P3/4HB-18; see text) for amorphous P4HB and P3/4HB-18 at 75.4 MHz as a function of temperature.

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 Dekmezian *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,^{9,18} as recently shown for PHO.^{11,17} ^{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,⁷ 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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