Unambiguous Determination of the ^{13}C and ^{1}H NMR Stereosequence Assignments of Polylactide Using High-Resolution Solution NMR Spectroscopy

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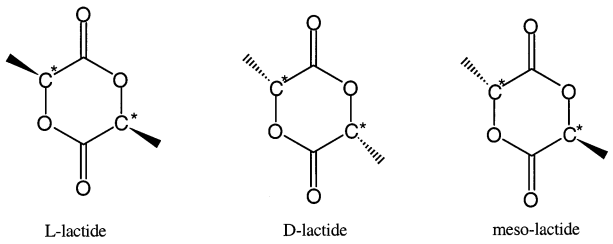
ABSTRACT: Polylactide (PLA) is a biodegradable polyester formed by the ring-opening polymerization of lactide, the cyclic dimer of lactic acid. Many of the physical properties of PLA are influenced by the amount and distribution of the *R* and *S* stereocenters in the polymer chain. NMR spectroscopy is the most common technique used to determine the stereosequence distribution of the polymer. The correct determination of the stereosequence distribution is contingent upon the assignment of the peaks in the NMR spectrum to specific stereosequences. Recently, alternative assignments to the commonly accepted stereosequence assignments have been proposed. If any of these alternative assignments are found to be correct, it would invalidate the conclusions of much of the previous work in understanding the kinetics of lactide polymerization and determining the stereoselectivity of new catalysts. The most significant problem is reconciling the commonly accepted peak assignments, which were based upon statistical probabilities, with contradictory connectivity data observed in a HETCOR NMR experiment. To describe the directionality in PLA, we have modified the nomenclature used to describe directionality in peptides and proteins. For PLA, the end containing the carboxylic group is referred to as the C terminus, and the end with the hydroxyl group is the O terminus. We had previously proposed that the central pairwise relationship (isotactic or syndiotactic, denoted *i* or *s*) in the ^{1}H NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the O terminus and that in the ^{13}C NMR spectrum it is determined by the central pairwise relationship of the stereocenter in the lactic acid unit attached to the C terminus. One- and two-dimensional NMR techniques, in combination with selective isotopic labeling, were used to show that this relationship is correct and that the commonly accepted assignments are correct. In addition, all of the nondegenerate resonances in the ^{1}H and ^{13}C NMR spectrum of polylactide at the tetrad stereosequence level have been assigned.

Introduction

Poly(lactide) (PLA) is a biodegradable aliphatic polyester which decomposes into carbon dioxide and water in a typical compost environment.[1] PLA is formed by the ring-opening polymerization of lactide, the cyclic dimer of lactic acid.[2,3] Lactic acid has two possible configurations, *R* and *S*, depending upon the arrangement of substituents around the chiral carbon. There are therefore three possible configurations of lactide: *RR*, *SS*, and *RS*. An *RR* configuration is referred to as D-lactide, *SS* is referred to as L-lactide, and *RS* is referred to as *meso*-lactide, as shown in Scheme 1. A mixture of equal amounts of D- and L-lactide is referred to as racemic or DL-lactide. Isotactic poly(D-lactide) and poly(L-lactide) are prepared from D- and L-lactide, respectively.[4−7]

Many of the physical properties of PLA are influenced by the amount and distribution of the *R* and *S* stereocenters in the polymer chain.[8] The distribution is determined by the relative probability that a specific sequence of stereocenters will be present in the polymer (i.e., *RRRR*, *RSSR*, etc.). Polymers with high stereoregularity can form highly crystalline polymers. For example, isotactic poly(L-lactide) crystallizes at a faster rate and to a higher degree than poly(L-lactide) that was synthesized using small amounts of D-lactide and/or *meso*-lactide.[4−7] Chains of poly(L-lactide) and poly(D-lactide) can form a crystalline stereocomplex, which has a higher melting point than poly(L-lactide).[9,10] Both racemic lactide and *meso*-lactide form an amorphous polymer using nonstereoselective catalysts.[7,11,12]

Scheme 1



The final stereosequence distribution of the polymer is dependent upon the catalyst used in the polymerization process and the stereochemical composition of the feed.[13−16] This has led to extensive research into the design of new stereoselective catalysts for synthesizing

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PLA with well-defined microstructures. Coates and co-workers have recently demonstrated the ability to synthesize both syndiotactic (-*RSRSRSRS*-) and heterotactic (-*RRSSRRSS*-) poly(lactic acid) using catalysts which exhibit precise stereochemical control.[16,17] In addition, Smith, Baker, and co-workers have designed a catalyst that has a strong preference for isotactic addition.[14] Using racemic lactide as a starting material, the resulting polymer contained stereoregular chains composed of either *R*-lactic acid or *S*-lactic acid. However, Coates and co-workers have challenged the interpretation of the structure, claiming that the actual polymer is composed of alternating segments that contain long sequences of either *R* or *S* stereocenters.[15]

It is essential to know the correct stereosequence distribution of the polymer in order to correctly determine the stereoselectivity of a catalyst. For PLA, the stereosequence of the polymer is normally determined using NMR spectroscopy.[18−27] In the NMR spectrum of a given polymer, the observed resonances can be assigned to stereosequence combinations in the polymer, although why the different chemical shifts arise is still unknown.[26] It is common in the PLA literature to designate the assignments as various combinations of "*i*" isotactic pairwise relationships (-*RR*- and -*SS*-) and "*s*" syndiotactic pairwise relationships (-*RS*- and -*SR*-). In the NMR spectra, the diads -*RR*- and -*SS*- are indistinguishable and have the same chemical shifts, as do the diads -*RS*- and -*SR*-. The number of resonances that can be observed in the NMR spectrum of a particular polymer indicates the stereosequence sensitivity. For stereosequence sensitivity of length *n*, there are $2^{(n-1)}$ possible combinations of pairwise relationships that can be observed in the NMR spectra.[26] We have shown that it is possible to differentiate chemical shifts for sequences that contain up to eight stereocenters.[22,23] This would correspond to octad stereosequence sensitivity, and there would be seven pairwise relationships between the individual units. As an example, for a polymer containing the *RRSSRRSS* subunit, the observed octad stereosequence would be *isisisi*. Because of either insufficient resolution, overlap of chemical shifts, or probability of stereosequence formation, not all the possible stereosequence combinations are observed in the NMR spectra.[26] Most of the determinations of the stereosequence probabilities have used the methine carbon for ^{13}C NMR and the methine proton for ^{1}H NMR. It is possible to determine the stereosequence distribution using the methyl and carbonyl carbons, or the methyl proton, but the NMR spectra are much more difficult to interpret.

Previous assignments of peaks in the NMR spectrum to particular stereosequences were made by using statistical methods to determine the probabilities for each possible stereosequence and comparing them to either the integrated intensity or the height of the peaks.[2,11,12,18,22,27,28] Kricheldorf et al. originally used the methine resonance in ^{1}H and ^{13}C NMR spectra to identify the stereosequence distribution of a number of PLA samples prepared under various reaction conditions.[2,27] The resolution in their spectra was limited, and only tetrad stereosequence distributions were evaluated. We have recently extended the methine stereosequence assignments in PLA to the hexad level and developed a method for quantitating the amount of L-, D-, and *meso*-lactide in PLA.[22,23] There was an excellent correlation between the anticipated intensities based upon statistical models and experimental data for polymers synthesized using various combinations of D-, L-, and *meso*-lactide.

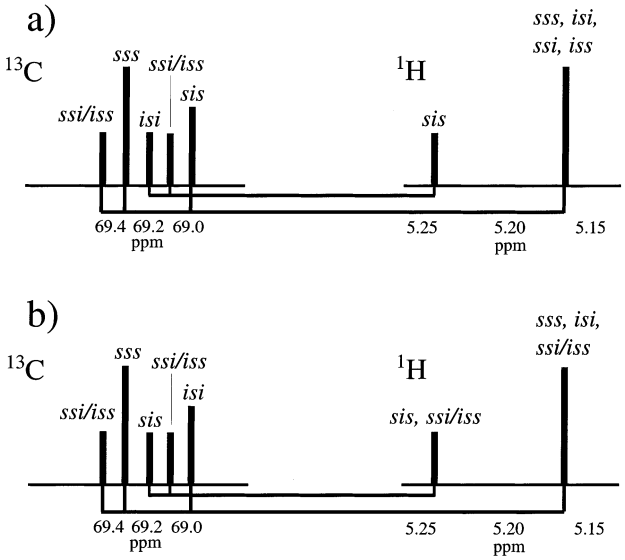

Figure 1. Comparison of (a) previously accepted and (b) newly proposed tetrad stereosequence assignments for the methine carbon and proton in PLA synthesized using *meso*-lactide. Lines between peaks in the ^{1}H and ^{13}C NMR spectra indicate connectivity observed in the HETCOR NMR spectra.

The accepted tetrad stereosequence assignments presented by Kricheldorf for both ^{1}H and ^{13}C NMR resonances in PLA have been recently questioned on the basis of results from ^{1}H−^{13}C heteronuclear correlation (HETCOR) experiments.[20,21] New assignments were proposed on the basis of the HETCOR results. A comparison of the Kricheldorf stereosequence assignments in PLA with the newly proposed assignments is shown in Figure 1.

It is imperative that the assignments based on the HETCOR NMR spectra and the assignments based on statistical probabilities be reconciled. We suspected that the interpretation of the HETCOR data was incorrect and proposed an alternative interpretation.[25] In this interpretation, the Kricheldorf assignments (based upon the statistical probabilities) are used. In our interpretation, it is also necessary to define the *directionality* of the polymer. We have modified the nomenclature used to describe directionality in peptides and proteins. For PLA, the end containing the carboxylic group is referred to as the C terminus, and the end with the hydroxyl group is the O terminus. In our interpretation, the central pairwise relationship (isotactic or syndiotactic, denoted *i* or *s*) in the ^{1}H NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the O terminus, and in the ^{13}C NMR spectrum, it is determined by the central pairwise relationship of the stereocenter in the lactic acid unit attached to the C terminus (Figure 2). Moreover, the polymer will always be depicted with the C terminus to the left and the O terminus to the right. An example of this assignment is shown with the sequence -*RS**S**RR*-, or -*sisi*-, where the center lactic acid subunit (in bold and underlined) is observed in both the ^{1}H and ^{13}C NMR spectra. In this assignment, the tetrad sequence for the ^{13}C is *sis* and for the ^{1}H is *isi*. We have shown that this assignment is consistent with the HETCOR NMR results.[20,21] However, no experimental data were provided to confirm this interpretation. Since then, there have been

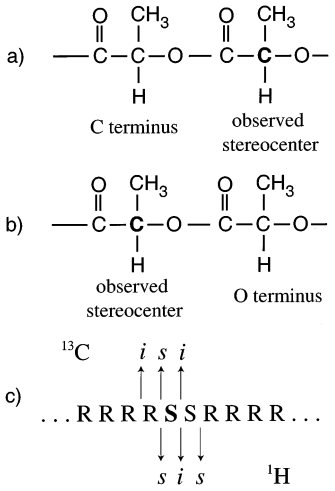

Figure 2. Diagram illustrating the alternative interpretation of ^{1}H and ^{13}C stereosequences: (a) central pairwise relationship determined by lactic acid connected to C terminus; (b) central pairwise relationship determined by lactic acid connected to O terminus; (c) direction of central pairwise relationship of ^{1}H and ^{13}C resonances.

several other alternative assignments proposed for interpreting the NMR data.[19,21]

In this paper, we attempt to resolve all of the uncertainty about the assignments of the peaks in the ^{1}H and ^{13}C NMR spectra of PLA. We show that our explanation of the HETCOR data, in which the central pairwise relationship in the ^{1}H NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the O terminus and in the ^{13}C NMR spectrum it is determined by the central pairwise relationship of the stereocenter in the lactic acid unit attached to the C terminus, is correct. Both one-dimensional ^{1}H and ^{13}C NMR experiments along with HMQC[29] (1 bond) and HMBC[30] (2−3 bonds) two-dimensional experiments were used to unambiguously assign the observed ^{1}H and ^{13}C stereosequences for a given central lactic acid unit in PLA. These experiments both confirmed the initial stereosequence assignments of Kircheldorf and resolved the inconsistencies between these stereosequence assignments and the HETCOR spectra.

Experimental Section

Solution NMR Spectroscopy. The ^{13}C and ^{1}H one-dimensional NMR spectra and 2D HMQC NMR spectrum of PLA were acquired on a Varian 500 MHz NMR spectrometer operating at 125.7 and 499.9 MHz, respectively. The HMBC NMR spectrum was acquired on a Varian 600 MHz NMR spectrometer operating at 600.5 MHz for ^{1}H and 151.0 MHz for ^{13}C.

The ^{13}C NMR spectra of a 10% solution of PLA in $CDCl_3$ were acquired using proton decoupling. A total of 64 000 data points were acquired at a spectral width of 30 kHz, corresponding to an acquisition time of 2.1 s. The recycle time was set at 1 s, and 4000 scans were averaged. The ^{1}H NMR spectra of a 1% solution of PLA in $CDCl_3$ were acquired with the methyl protons decoupled from the methine protons (homonuclear decoupling) during the acquisition time. Sixty-four scans were acquired, each with 40 000 data points at a spectral width of 10 kHz, corresponding to an acquisition time of 4 s. A pulse delay of 1 s was used.

The HMQC spectrum was acquired at a ^{1}H frequency of 499.869 MHz. A total of 1024 data points were acquired in the proton dimension with a spectral width of 4.2 kHz. One transient was acquired for 1024 t_1 increments to generate the ^{13}C dimension.

The HMBC spectrum was acquired at a ^{1}H frequency of 600.476 MHz. A total of 2048 data points were acquired in the proton dimension with a spectral width of 4.8 kHz. Sixteen transients were acquired for 512 t_1 increments to generate the ^{13}C dimension.

Preparation of Polylactide Samples. Fully ^{13}C-labeled L-lactide was prepared from ^{13}C-labeled lactic acid (Isotec) by standard procedures.[22] PLA samples synthesized from 5% unlabeled l-lactide in 95% D-lactide and 5% fully ^{13}C-labeled L-lactide in 95% D-lactide and 5% fully ^{13}C-labeled L-lactide in 95% *meso*-lactide were polymerized using tin octanoate as an initiator in toluene at 70 °C for 18 h.

Results and Discussion

The assignment of peaks in the solution NMR spectra to specific stereosequences in PLA must satisfy two criteria. First, the integrated intensities of the peaks assigned to each stereosequence must agree with statistical probabilities. Second, the assignment must also be consistent with NMR data, such as connectivities observed in a HETCOR experiment. Our goal was to find an explanation that was consistent with both statistics and NMR. We had previously proposed that the central pairwise relationship in the ^{1}H NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the O terminus and that in the ^{13}C NMR spectrum it is determined by the central pairwise relationship of the stereocenter in the lactic acid unit attached to the C terminus, as illustrated in Figure 2.

Our first step was to synthesize PLA samples with known microstructures to confirm the stereosequence assignments proposed by Kricheldorf.[27] Two approaches were taken. First, PLA synthesized using primarily L-lactide with small amounts of D-lactide generated stereosequence distributions that consisted of isolated *RR* segments, or defects, connected to long sequences of *S* stereocenters. It was then possible to accurately predict the stereosequence distribution of this polymer, without having to specify the statistics used to predict peak intensities. The other approach was to synthesize PLA that incorporates ^{13}C-labeled L-lactide in order to selectively observe those lactic acid subunits that contain the isotopic label. This approach is extremely powerful, because signals arising from *S* stereocenters could be differentiated from signals from *R* stereocenters. This information can be used to predict the stereosequences arising only from *S* stereocenters. In addition, the ability to differentiate the signals from *R* vs *S* stereocenters can be used in two-dimensional techniques for assigning resonances due to particular stereosequences and for determining the directionality of the ^{1}H and ^{13}C stereocenters.

The first step in assigning the spectra was to synthesize PLA using 5% unlabeled L-lactide and 95% unlabeled D-lactide. This polymer can ideally be divided into segments which contain 40 stereocenters, with two *S* stereocenters bordered on each side by 38 *R* stereocenters. As expected for PLA synthesized using predominantly D-lactide, the *iii* peak intensity should be dominant due to the large regions of isotacticity in the polymer. Out of the 40 stereocenters, 35 of them will produce signals from *iii* stereorelationships (87.5%). In the region around the pair of *S* stereocenters (generated by the L-lactide), syndiotactic relationships will be observed, resulting in 2 *isi* (5%), 1 *sii* (2.5%), 1 *iis* (2.5%), and 1 *sis* (2.5%) stereorelationships. Since the integrated intensity of each resonance is directly proportional to the percentage of stereocenters producing that signal,

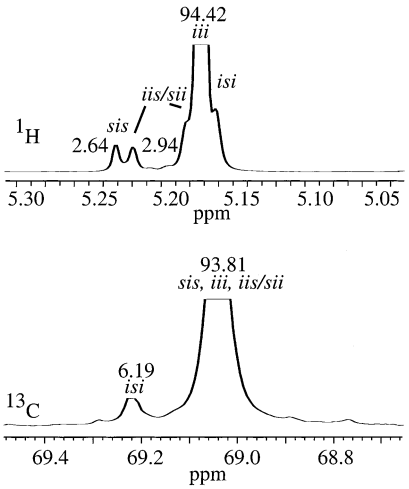

Figure 3. ^{1}H and ^{13}C solution NMR spectra of PLA synthesized using 5% L-lactide and 95% D-lactide.

the integration values for the resonances in the ^{1}H and ^{13}C NMR spectra can be directly compared with the expected intensity of each resonance based upon the probability that the stereosequence occurs in the polymer.

The ^{1}H and ^{13}C NMR spectra of PLA synthesized using 5% L-lactide and 95% D-lactide are shown in Figure 3. In the ^{1}H spectrum, overlap of the *isi* resonance with the *iii* resonance makes direct integration of the *isi* resonance impossible. Similarly, in the ^{13}C spectrum, overlap of the *sis* resonance with the *iii* resonance makes direct integration of the *sis* resonance impossible. In the ^{1}H NMR spectrum, the *sis* resonance at 5.24 ppm gives an integrated intensity of 2.64%, which corresponds well with the expected value of 2.5% from the accepted stereosequence assignments. Integration of the resonance at 69.21 ppm (the *isi* resonance) in the ^{13}C NMR spectrum gives a value of 6.19%, in comparison with the expected value of 5%. One of the assumptions that was made in interpreting the results of this experiment is that the *S* stereocenters from the L-lactide had at least four *R* stereocenters from D-lactide on either side. We had previously determined that there is a slight preference for syndiotactic addition with this catalyst.[22] However, the concentration of L-lactide was low enough to minimize any effect due to syndiotactic preference. Also, analysis of PLA which was synthesized using primarily D-lactide and small amounts of L-lactide that was ^{13}C-labeled in the carbonyl position showed only two peaks in the carbonyl region due to the ^{13}C labels, which confirms that almost all the pairs of *S* stereocenters are surrounded on either side by several *R* stereocenters (spectrum not shown).

These results are consistent with the statistical assignments but do not agree with the assignments based upon the HETCOR NMR data.[20] The resonance at 69.21 ppm in the ^{13}C NMR spectrum, assigned from the HETCOR NMR data as *sis*, must be *isi* for this polymer because the observed intensity (~6%) is a factor of 2 larger than that predicted for *sis* (2.5%). In addition, the resonance at 5.24 ppm in the ^{1}H NMR spectrum was assigned to both *sis* and *sii*/*iis* from the HETCOR data. However, the expected intensity of the sum of the *sis* and one-half the *sii*/*iis* resonances is ~5%. The normalized integrated intensity of the resonance in question is 2.64%, indicating that this resonance must be due only to either *sis* or *sii*/*iis*. These results are inconsistent with the HETCOR assignments for the ^{1}H and ^{13}C NMR spectra of PLA but are consistent with the Kricheldorf assignments.

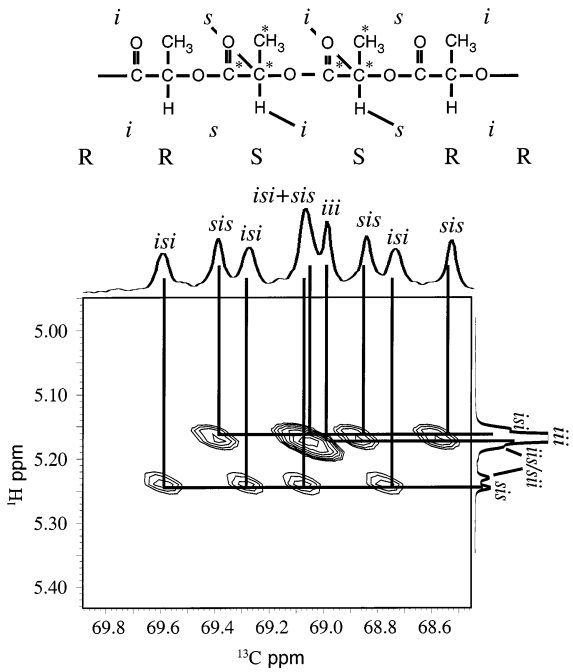

Figure 4. ^{1}H−^{13}C HMQC spectrum of PLA synthesized using 5% fully ^{13}C-labeled L-lactide and 95% D-lactide.

The second step in assigning the spectra was to repeat the synthesis of PLA using 5% L-lactide and 95% D-lactide, except fully ^{13}C-labeled L-lactide was substituted for unlabeled L-lactide. In this experiment, the peaks from the *S* stereocenters can be easily identified, as they should have both an increased peak intensity due to the ^{13}C label and a splitting due to ^{13}C−^{13}C *J* coupling to the carbonyl and methyl carbons. We then used a two-dimensional ^{1}H−^{13}C correlation experiment to determine which resonance in the ^{13}C spectrum is correlated to which resonance in the ^{1}H spectrum. We chose to use an HMQC experiment instead of a traditional HETCOR experiment because of the increase in sensitivity that arises in the HMQC experiment from direct detection of the proton signal.

Figure 4 shows the methine correlation peak region of the ^{1}H−^{13}C HMQC spectrum of PLA synthesized using 5% fully ^{13}C-labeled L-lactide and 95% D-lactide. The stereosequence for L-lactide carbons bracketed on either side by a D-lactide is -*RR**SS**RR*-, or -*isisi*-. The *S* stereocenters arising from the ^{13}C-labeled lactide (denoted in bold and underlined) should produce only *isi* and *sis* resonances, and these two resonances are observed in the ^{1}H dimension. In the ^{13}C dimension, the signal from each *S* stereocenter is split into a doublet of doublets due to ^{13}C−^{13}C *J* coupling of the methine carbon to the ^{13}C labels in the methyl and carbonyl carbons. For the methine region, ^{13}C chemical shifts of 68.95 ppm for *sis* and 69.18 ppm for *isi* are expected on the basis of the results of the ^{1}H and ^{13}C NMR spectra shown in Figure 3. Four correlation peaks, centered at 69.18 ppm and corresponding to a doublet of doublets for *isi*, lie about 0.23 ppm further downfield in the ^{13}C dimension than the four peaks for *sis*. If the stereosequence assignments proposed by Kricheldorf are used, correlation peaks between the ^{13}C *sis* doublet of doublets (centered at 68.95 ppm) and the ^{1}H *isi* peak (5.17 ppm) are observed, along with correlation peaks between the

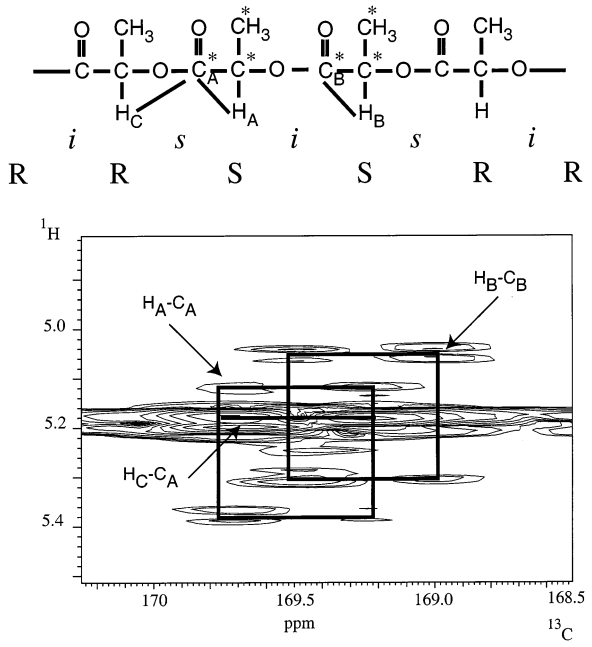

Figure 5. ^{1}H−^{13}C HMBC spectrum of PLA synthesized using 5% fully ^{13}C-labeled L-lactide and 95% D-lactide.

^{13}C *isi* doublet of doublets (centered at 69.18 ppm) and the ^{1}H *sis* peak (5.24 ppm). A large correlation peak due to the natural abundance *R*-lactide (*iii*) is present at 5.18 ppm in the ^{1}H dimension and 69.02 ppm in the ^{13}C dimension. The diagonal slant of the correlation peak resonances observed in Figure 4 is an artifact of the HMQC experiment.

The two-dimensional HMQC experiment on PLA synthesized using 5% fully ^{13}C labeled L-lactide and 95% D-lactide shown in Figure 4 shows that the assignments of the methine proton and carbon are different (i.e., ^{13}C is *sis* when ^{1}H is *isi* and ^{13}C is *isi* when ^{1}H is *sis*). This result also confirms the correlations observed in the previously reported HETCOR NMR experiment.[20] However, it is also consistent with our interpretation that the central pairwise relationship in the ^{1}H NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the O terminus and that in the ^{13}C NMR spectrum it is determined by the central pairwise relationship of the stereocenter in the lactic acid unit attached to the C terminus. The significant results of this experiment are that we can unambiguously identify the resonances due to *isi* and *sis* tetrad stereosequences and that we can confirm the results of the HETCOR NMR experiment. This experiment does not unambiguously assign the *isi* and *sis* tetrads.

The next step was to perform a heteronuclear multiple-bond correlation (HMBC) experiment on this sample to determine whether the central pairwise relationship in the ^{1}H NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the O terminus or whether it is determined by the central pairwise relationship of the stereocenter in the lactic acid unit attached to the C terminus. An HMBC experiment, which is a heteronuclear correlation experiment like HMQC, allows for only multiple bond (2−3) couplings to be observed. Figure 5 shows an expansion of the region corresponding to the correlation peak between the carbonyl carbon and methine proton in the ^{1}H−^{13}C HMBC spectrum of the same PLA sample (5% fully ^{13}C labeled L-lactide and 95% D-lactide) as in Figure 4. Three main correlation peaks are observed for this sample. Two sets of correlation peaks correspond to methine proton to carbonyl carbon connectivity within the same ^{13}C-labeled *S*-lactic acid monomer unit. The first correlation peak, centered at 5.17 ppm in the ^{1}H dimension and 169.2 ppm in the ^{13}C dimension, corresponds to an H_B−C_B (*isi*−*sis*) correlation peak. The second correlation peak, centered at 5.24 ppm in the ^{1}H dimension and 169.4 ppm in the ^{13}C dimension, corresponds to an H_A−C_A (*sis*−*isi*) correlation peak. Both sets of correlation peaks are split into doublets in both the ^{1}H and ^{13}C dimensions. The methine proton is coupled to the ^{13}C-labeled methine carbon, resulting in a doublet. The ^{13}C-labeled carbonyl carbon is coupled to the ^{13}C-labeled methine carbon, which also results in a doublet. We have not investigated stereosequence assignments for the carbonyl region in PLA. However, preliminary hexad level assignments of the carbonyl region in the ^{13}C NMR spectrum of PLA have been reported in the literature by Kasperzyck.[18] These assignments, in which the *isisi* hexad (*sis* tetrad) is located at 169.2 ppm and the *iisii*, *sisii*, *iisis*, and *sisis* hexads (*isi* tetrad) are located at 169.4 ppm, are consistent with the assignments in the HMBC spectrum. A third correlation peak is also observed between the methine proton of an unlabeled *R*-lactic acid unit and the carbonyl carbon of the neighboring labeled *S*-lactic acid unit. This correlation peak, centered at 5.17 ppm in the ^{1}H dimension and 169.4 ppm in the ^{13}C dimension, corresponds to an H_C−C_A (*isi*−*isi*) correlation peak. This is a three-bond coupling, which can be observed in an HMBC experiment. This correlation peak is different from the other two because the proton resonance is not split by ^{13}C−^{1}H coupling, since it is attached to an unlabeled *R* methine carbon.

The presence of this H_C−C_A correlation peak indicates that for ^{1}H stereosequences the central stereosequence relationship is determined by the chirality of the stereocenter attached to the O terminus, because H_C is located on the monomer unit attached to the C terminus of one of ^{13}C labeled *S*-lactic acid monomer units. For ^{13}C, the central stereosequence relationship is determined by the chirality of the stereocenter attached to the C terminus, because of the observance of the *isi*−*sis* correlation in the HMQC and HMBC spectra. This experiment also unambiguously assigns the ^{1}H *isi* tetrad stereosequence to the peak at 5.17 ppm. Since there can be only one signal from a ^{1}H corresponding to an *sis* tetrad, the peak that produces two signals at the same chemical shift in the HMQC experiment must correspond to an *isi* tetrad stereosequence. Although the assignment of this peak to the ^{1}H *isi* tetrad has been consistent throughout all of the proposed assignments, this experiment now provides definitive proof for it.

At this point our experimental results show the following: (1) experiments performed on PLA synthesized using 5% L-lactide and 95% D-lactide show that the statistical distributions are consistent with the previous stereosequence assignments but are inconsistent with the assignments based on the HETCOR connectivities; (2) HMQC experiments show that peaks corresponding to *isi* and *sis* stereosequences can be identified (but not differentiated from one another); (3) HMBC experiments show that the *isi* stereosequence has been unambiguously assigned in the ^{1}H NMR spectrum to the peak at 5.17 ppm; and (4) HMBC experiments also show that for ^{1}H stereosequences the central stereosequence relationship is determined by the

Table 1. Expected Relative Intensity (Adjusted To Compensate for Incorporation of Labeled Material) Arising from the ^{13}C-Labeled Stereocenters and the Unlabeled Stereocenters for PLA Synthesized Using 5% Fully ^{13}C-Labeled L-Lactide and 95% *meso*-Lactide

stereosequences from ^{13}C-labeled stereocenters	expected relative intensity/4	chemical shift	stereosequences from unlabeled stereocenters	expected relative intensity	chemical shift
sii	0.47	68.95/69.03	*iss*	0.13	69.4
ssi	0.31	69.08	*sss*	0.39	69.28
sis	0.16	68.98	*isi*	0.13	69.18
iii	0.16	69.0	*ssi*	0.13	69.08
iis	0.16	68.95/69.03	*sis*	0.26	68.98

chirality of the stereocenter attached to the O terminus and for ^{13}C the central stereosequence relationship is determined by the stereocenter attached to the C terminus. The final experiment that is needed to unambiguously assign the ^{1}H and ^{13}C spectra is one that will produce either an *isi* or *sis* tetrad stereosequence in the ^{13}C NMR spectrum, but not both. Since the ^{1}H *isi* and *sis* resonances have been assigned from the HMQC and HMBC experiments to peaks at 5.17 and 5.24 ppm, respectively, the definitive assignments of the ^{13}C *isi* and *sis* peaks will confirm that our alternative explanation of the HETCOR data is correct.

One experiment that will produce only *sis* tetrads and no *isi* tetrads is to synthesize PLA containing primarily *meso*-lactide (*RS*) with a small amount of fully ^{13}C-labeled L-lactide (*SS*). In this case, only four possible combinations of L-lactide in *meso*-lactide are possible: *RSSSRS*, *SRSSRS*, *RSSSSR*, and *SRSSSR*. There are therefore only five possible stereosequences that can arise from an *S* stereocenter generated by ^{13}C-labeled L-lactide: *sii*, *iis*, *sis*, *iii*, and *ssi*. **No peak corresponding to an *isi* tetrad should be observed from the ^{13}C-labeled *S* stereocenters.** In addition, because the central pairwise relationship in the ^{13}C NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the C terminus, only the *ssi* sequence should be observed.

The NMR spectra of PLA synthesized using (a) fully ^{13}C-labeled and (c) unlabeled 5% L-lactide and 95% *meso*-lactide are shown in Figure 6, along with (b) the NMR spectrum of poly(*meso*-lactide). The spectra in Figure 6b,c are almost identical, because the stereosequence distributions for 5% L-lactide/95% *meso*-lactide and for 100% *meso*-lactide are almost identical. The NMR spectrum in Figure 6a is very different than the spectra in Figure 6b,c. The reason is that the signals from the fully ^{13}C-labeled *S* stereocenters dominate the spectrum, which is similar to the situation observed in the one-dimensional ^{13}C NMR spectrum in Figure 4. In this experiment, single resonances will be observed for the methine carbon for unlabeled stereocenters that came from the *meso*-lactide, and a doublet of doublets will be seen for the fully ^{13}C-labeled *S* stereocenters in the methine carbons from the ^{13}C-labeled L-lactide, because of ^{13}C−^{13}C *J* coupling between the methine carbon and the methyl and carbonyl carbons. The spectrum in Figure 6a contains all of the information necessary to unambiguously assign the *sis* tetrad.

As a result of the splitting of the signals for the ^{13}C-labeled *S* stereocenters into a doublet of doublets, the spectrum of the labeled polymer spans a larger chemical shift range (68.5−69.5 ppm) than the unlabeled polymer (69.0−69.4 ppm). The center of each set of doublet of doublets corresponds to the expected chemical shift value for an unlabeled carbon. The stereosequences, relative intensities, and chemical shift values for the ^{13}C-labeled stereocenters and the unlabeled stereocenters are shown in Table 1.

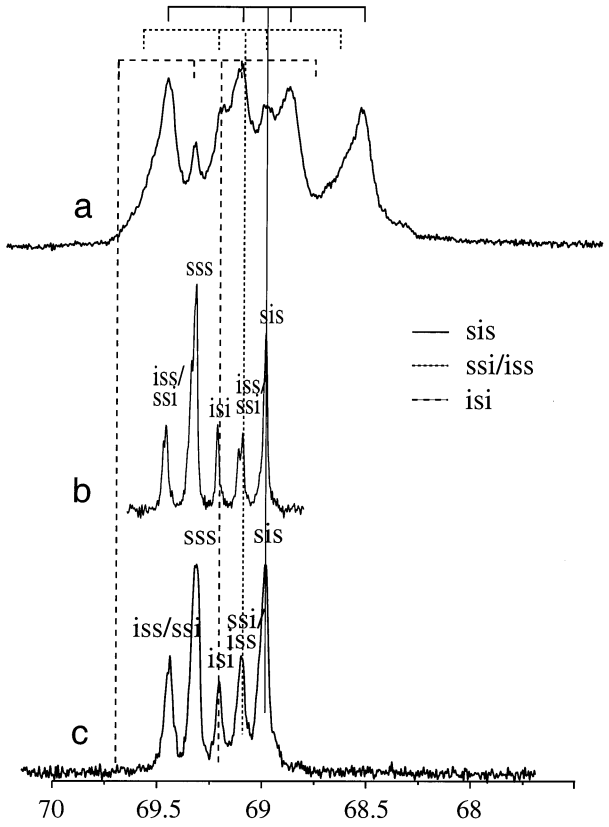

Figure 6. ^{13}C NMR spectra of PLA synthesized using (a) 5% fully ^{13}C-labeled L-lactide and 95% *meso*-lactide, (b) *meso*-lactide, and (c) 5% L-lactide and 95% *meso*-lactide.

The *sis* stereosequence is expected from both the unlabeled and labeled stereocenters. For the unlabeled *meso*-lactide component an *isi* stereosequence is expected, while for the ^{13}C-labeled stereocenters an *isi* stereosequence is not expected. In the unlabeled *meso*-lactide region, the *sis* resonance appears at 69.0 ppm and is not split, while in the ^{13}C-labeled L-lactide region, the *sis* resonance is centered at 69.0 ppm but is split into a doublet of doublets which extends ∼0.4 ppm in both directions, spanning the region from 68.6 to 69.4 ppm. In addition, all of the other resonances expected due to the ^{13}C-labeled L-lactide lie within 0.1 ppm of the *sis* resonance, making the total span of all resonances due to ^{13}C-labeled L-lactide from 68.5 to 69.5 ppm (Figure 6). This experiment definitively proves that the peak at 69.0 ppm in the ^{13}C NMR spectrum is due to *sis*. If the *isi* and *sis* stereosequence assignments were reversed, then the *sis* peak would be centered at 69.2 ppm and would span from 68.8 to 69.6 ppm. The solid line in Figure 6 shows where the *sis* peak would be centered, the short-dashed line shows where the *iss*/*ssi* peak would be centered, and the long-dashed lines show where the *isi* peak would be centered and where the furthest peak in that doublet of doublets should be.

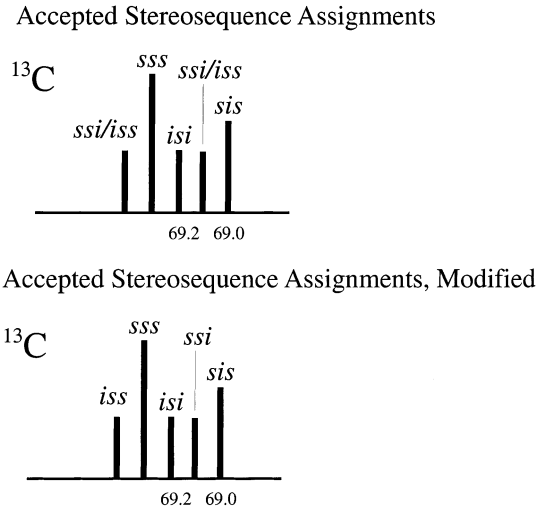

Figure 7. Modified ^{13}C stereosequence assignments for poly(*meso*-lactide), showing definitive assignment of *iss* and *ssi* resonances.

Since there is no resonance at 69.6 ppm, the assignment of the peak at 69.0 to *sis* and the peak at 69.2 to *isi* must be correct.

It is also possible to definitively assign the *iss*/*ssi* stereosequences to specific resonances in the ^{13}C NMR spectrum. For a polymer that is synthesized with no isotopically labeled material, both the *iss* and *ssi* resonances will always have equal probability and therefore cannot be distinguished in the NMR spectrum. For PLA synthesized using 5% fully ^{13}C-labeled L-lactide and 95% *meso*-lactide, both the *ssi* and *iss* stereosequences are expected from the large amount of *meso*-lactide, and these resonances will not be split in the NMR spectrum. When PLA is synthesized using 5% fully ^{13}C-labeled L-lactide and 95% *meso*-lactide, only the *ssi* stereosequence will be split into a doublet of doublets (Table 1) because of $^{13}C-^{13}C$ *J* coupling to the methyl and carbonyl carbons. No peak due to *iss* will be observed from the ^{13}C-labeled stereocenters. If the *ssi* resonance is centered at 69.1 ppm, the doublet of doublets will overlap the doublet of doublets generated by the *iii*, *iis*, *sii*, and *sis* stereosequences, and the spectrum will span from about 68.7−69.5 ppm (Figure 6). If the *ssi* resonance occurs at 69.4 ppm, the doublet of doublets will span from 69 to 69.8 ppm. The ^{13}C NMR spectrum in Figure 6a has no resonance above 69.5 ppm, indicating that the resonance at 69.1 ppm in Figure 6b is due to *ssi*, while the resonance at 69.4 ppm is due to *iss*. The definitive assignments of the *sis*, *isi*, *sss*, *iss*, and *ssi* stereosequence assignments in the ^{13}C NMR spectrum of *meso*-lactide are shown in Figure 7. This interesting result could only be obtained by studying ^{13}C-enriched samples.

We can use the HETCOR data[19−21] presented in several recent studies to further assign the ^{1}H NMR spectrum of poly(*rac*-lactide). The HETCOR data show a correlation peak between the *isi* peak in the ^{13}C NMR spectrum and between the *sis* and *iis*/*sii* peaks. We were not able to assign the *iis*/*sii* peaks in the past because both stereosequences have the same statistical probabilities. However, we do know that for poly(*rac*-lactide) the *isi* peak in the ^{13}C NMR spectrum should have correlation peaks with the *sis* and *sii* peaks in the ^{1}H NMR spectrum. Since these correlation peaks are observed at 5.22 and 5.24 ppm in the ^{1}H NMR spectrum, the assignments in Figure 8 for the ^{1}H spectrum are

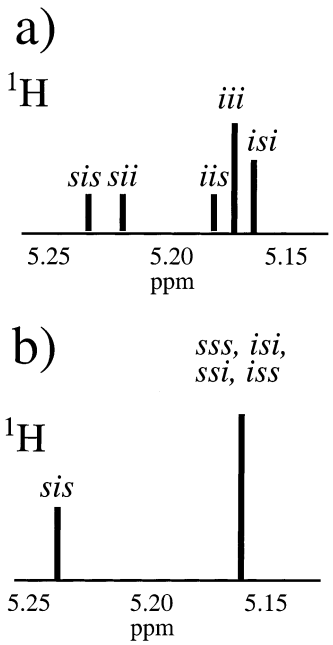

Figure 8. Assigment of the ^{1}H stereosequence assignments for PLA synthesized using (a) *rac*-lactide and (b) *meso*-lactide. The additional assignment of the *iis* and *sii* peaks based on the HETCOR NMR data is shown.

now complete. We have now completely assigned all of the nondegenerate resonances in the ^{1}H and ^{13}C NMR spectrum of polylactide at the tetrad stereosequence level.

Conclusion

We have used a combination of two-dimensional NMR experiments and selective ^{13}C labeling to assign both the ^{1}H and ^{13}C NMR spectrum of the methine proton and carbon in PLA at the tetrad stereosequence level. The *isi* and *sis* stereosequence assignments, for which HETCOR NMR and statistical distributions gave contradictory results, have been resolved. The interpretation that the central pairwise relationship (*i* or *s*) in the ^{1}H NMR spectrum is determined by the stereocenter in the lactic acid unit attached to the O terminus and that in the ^{13}C NMR spectrum it is determined by the central pairwise relationship of the stereocenter in the lactic acid unit attached to the C terminus is consistent with all NMR and statistical data.

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