Enzymatic Polymerization of Poly(ϵ-CL) Containing an Ethyl Glucopyranoside Head Group: An NMR Study

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The usefulness of the 2D-NMR techniques in the structure determination of the oligo ϵ-caprolactone ethyl glucopyranoside (oilgo(ϵ-CL) EGP) conjugate, synthesized by a lipase-catalyzed regioselective reaction, is described. Data from a ^{13}C–^{13}C COSY NMR spectrum of the ethyl glucopyranoside is used for complete and unambiguous assignments of the carbon resonances in the ^{13}C NMR spectrum of the anomeric mixture of EGP. A comparison of the DEPT-135 spectrum of the product oilgo(ϵ-CL) EGP conjugate with that of the starting material, ethyl glucopyranoside (EGP), led to the conclusion that the lipase-catalyzed ring-opening of ϵ-CL initiated by a multifunctional initiator, EGP, proceeded in a regioselective fashion. Also, a detailed analysis of the NMR data has allowed determination of the site for lipase-catalyzed ring-opening polymerization of ϵ-CL. Our study suggests that among the possible four initiation sites in EGP only the hydroxyl group on carbon C-6 took part in the initiation process.

Index Headings: **Lipase; Ethyl glucopyranoside; ϵ-Caprolactone; Ring-opening polymerization; Two-dimensional NMR; ^{13}C NMR; Regioselectivity; Multifunctional initiator; Site of reaction; Caprolactone; Glucopyranoside.**

INTRODUCTION

Amphiphilic compounds find important applications in industry and medicine as surface-active agents.[1] Polymers bearing sugar residues have been reported to be of great importance as pharmacological and biomedical materials. The sugar groups in these materials are reported to play an important role in these applications. Therefore incorporation of sugar groups in polymer chains is an important thrust area in synthesis of amphiphilic polymers. Vinyl monomers with sugar groups such as glucosylethyl methacrylate, alkyl, or aryl 6-*O*-acryl-α-D-glucopyranosides that can be subsequently polymerized have been prepared.[2,3] However, selective modification of one out of several hydroxyl groups in sugars has been a difficult challenge to synthetic chemists.[4–6] The chemical methods employed for this task are tedious and involve several protection/deprotection steps that frequently require isolation/purification at each step.[4–7] Enzymatic methods have increasingly been utilized for preparation of selectively modified sugars.[8–17] Sugars are soluble only in very polar solvents, viz., pyridine, dimethylformamide (DMF), and dimethylsulfoxide (DMSO), due to the increased number of hydroxyl functional groups in them. Enzyme-catalyzed transesterifications using activated esters for preparation of monoacylated sugars have been reported in DMF and pyridine. However, yields reported from these reactions are low.[8–16] Moreover, using these polar solvents prevents widespread use of such reaction schemes. Also, unfortunately, many enzymes are catalytically inactive in these solvent media.[18] Direct enzymatic esterifications of sugars with fatty acids in aqueous media have also been reported to give low yield and selectivity.[19] Use of alkyl pyranosides offers advantages due to their increased solubility in less polar solvents and higher reactivity. Interestingly, a dramatic increase in the reactivity of ethyl α-D-glucopyranoside as compared to either glucose or methyl α-D-glucopyranoside, in lipase-catalyzed esterification with dodecanoic acid, was reported by Bjorkling and co-workers.[20,21] The increased reactivity of the ethyl glucopyranoside (EGP) was attributed to higher solubilities of the reactants in one another. They also reported regioselective acylation of ethyl glucopyranoside, in bulk, for production of *n*-alkyl 6-*O*-acyl-α-D-glucopyranosides. Although high conversions were obtained, contamination by 2–14% of the diester was observed.[20,21]

In addition to the difficulties encountered in modification of sugars, additional tasks exist in conclusive identification of these compounds. Structure determination of selectively protected sugars has been a challenge because the proton and carbon-13 NMR spectra of these compounds are often complicated due to (1) the overlapping of several resonances, and (2) the presence of anomeric mixtures in this class of compounds.

We recently reported a one-pot highly regioselective synthesis of oligomeric ϵ-caprolactone (oligo(ϵ-CL)) bearing an ethyl glucose head group by ring-opening polymerization of ϵ-CL initiated by ethyl glucopyranoside and catalyzed by lipase from procine pancreas (Scheme I).[22] We report here the application of one- and two-dimensional (1D and 2D) NMR techniques for structure elucidation of the product. We also discuss how the one- and two-dimensional NMR techniques are useful in identifying the key reaction sites in this reaction scheme.

EXPERIMENTAL

Materials and Methods. Analytical-quality D-glucose was purchased from Sigma Chemical Company and was dried in a vacuum oven at 40 °C overnight prior to use. D-Glucose-$^{13}C_6$ (99% ^{13}C) was purchased from Aldrich Chemical Company and was used as received. ϵ-Caprolactone was also purchased from Aldrich and was distilled at 97–98 °C over CaH_2 at 10 mm of Hg prior to its use. Porcine pancreatic lipase (PPL, type II) was purchased from Sigma (25% protein, specified activity 61

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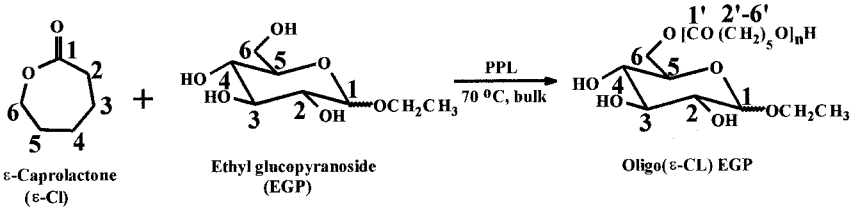

SCHEME I.

units/mg protein) and was stored at 4 °C. One unit is defined as the amount of protein that hydrolyzes 1 microequivalent of fatty acid from triacetin in 1 h at pH 7.4. Prior to use, lipase was dried over P_2O_5 overnight in a vacuum desicator at 0.1 mm of Hg. Deuterated NMR solvents were purchased from Aldrich and were used as received.

NMR Measurements. One- and two-dimensional NMR spectral data were collected by using 250 and 500 MHz NMR instruments. ^{1}H-NMR spectra were recorded on a Bruker ARX-250 spectrometer at 250 MHz. The NMR sample solutions were prepared with a concentration of 4% w/v in dimethylsulfoxide-d_6. The instrumental operating parameters were as follows: temperature 310 K, pulse width 4.9 μs (30° pulse), 32 KW data points, 3.17 s acquisition time, 1 s relaxation delay, and 16 transients. ^{13}C-NMR spectra were recorded at 62.9 MHz on a Bruker ARX-250 spectrometer. Samples were prepared in dimethylsulfoxide-d_6. Chemical shifts in parts per million (ppm) were referenced relative to DMSO-d_6 as an internal reference at 39.7 ppm. Following are the experimental parameters for ^{13}C NMR data acquisition: 10% w/v in DMSO-d_6, 310 K, pulse width 4.9 μs (30°), 64 KW data points, 1.638 s acquisition time, and 1 s relaxation delay. The number of transients varied from 15 000–18 000.

^{13}C–^{13}C COSY Experiment. The 2D ^{13}C–^{13}C COSY spectral data were acquired on the DRX 500 MHz NMR instrument by using the pulse sequence that was described by Aue et al.[23] The sample was prepared in the deuterated-DMSO. The following parameters were used for collecting 2D-NMR data: We accumulated 512 increments in t_1 and 2048 data points for each free induction decay (FID) in t_2. Sixty-four scans were collected for each FID with a relaxation delay of 1 s. The carbon-13 $\pi/2$ pulse width was 11.8 μs for ^{13}C NMR observation at 125.768 MHz. Quadrature detection was used, FIDs were multiplied by a sine function prior to Fourier transformations, and t_1 was zero-filled once to 1024 w to yield a processed data matrix of 1024 w × 1024 w (reals only). The spectral width was 9328 Hz in both dimensions. The spectrum was symmetrized.

Preparation of Ethyl Glucopyranoside. The compound was prepared according to the procedure described elsewhere.[20] In a typical experiment, in an oven-dried 250 mL round-bottom flask containing 25 g of absolute ethanol (0.55 mol), 10 g of vacuum-dried D-glucose (0.055 mol) and ion exchange resin Dowex-50 H^+ (2.5 g) were added. The reaction was then refluxed (for 20 h) until most of the glucose was dissolved. The reaction was then allowed to cool to room temperature and it was filtered. Ethanol was evaporated *in vacuo* to obtain ethyl glucopyranoside (12.65 g, > 98% yield) as a light yellowish viscous oil. The crude product was found to be a 2.1:1 mixture of α/β anomers, containing approxmately 1% unreacted glucose. The anomeric ratio of the product was calculated from the peak areas of the doublets at 4.65 and 4.15 ppm corresponding to the proton on C-1 in the α and the β anomer, respectively. Pure ethyl glucopyranoside was obtained after purification by column chromatography over silica gel (100–120 mesh) with the use of chloroform/methanol (9:1) mixture as eluent. Pure ethyl glucopyranoside, 10.30 g, was obtained as a colorless viscous oil.

Preparation of Ethyl Glucopyranoside-$^{13}C_6$. The procedure for the preparation of ^{13}C-labeled ethyl glucopyranoside was similar to the one described above for ethyl glucopyranoside except that 100 mg (0.0005 mol) glucose-$^{13}C_6$ and proportional quantities of reagents were used. The crude product was purified by column chromatography to give 90 mg of pure ethyl glucopyranoside-$^{13}C_6$. The ^{1}H-NMR spectrum of ^{13}C-labeled compound was similar to that of ethyl glucopyranoside, but the proton-decoupled ^{13}C-NMR spectrum showed resolved multiplets due to ^{13}C–^{13}C coupling.

Enzyme-Catalyzed Ring Opening of ϵ-Caprolactone by Ethyl Glucopyranoside. The synthesis was carried out as described elsewhere.[22] In a typical run, 200 mg of ethyl glucopyranoside and 500 mg of dry porcine pancreatic lipase (0.1 mm Hg, 25 °C, overnight) were transferred into an oven-dried 20 mL reaction vial under an argon atmosphere. The vial was immediately stoppered with a rubber septum and purged with argon. We added 0.8 mL of ε-caprolactone via syringe under argon atmosphere. The reaction vial was then placed in a constant temperature oil bath maintained at 70 °C for 96 h. In addition, a control reaction was set up in the same bath. The control sample was prepared in a similar way, except that PPL was not added. The reaction was then quenched by removing the enzyme with a vacuum filtration process (glass-fritted filter, medium-pore porosity). The enzyme was washed 3–4 times with 10 mL chloroform. All the filtrates were combined and the solvent was removed *in vacuo*. The final product was characterized by using its ^{1}H- and ^{13}C-NMR spectra.

RESULTS AND DISCUSSION

Proton NMR Spectral Analysis of Starting Materials: ϵ-CL and EGP. First we discuss the common features in one-dimensional proton NMR spectra of ε-CL and EGP (Figs. 1 and 2a, respectively). EGP initiated ring-opening polymerization of ε-CL in bulk catalyzed by porcine pancreatic lipase was carried out (see Experimental section). In the ^{1}H NMR spectrum of ε-CL the methylene protons next to the oxygen (labeled as 6 in

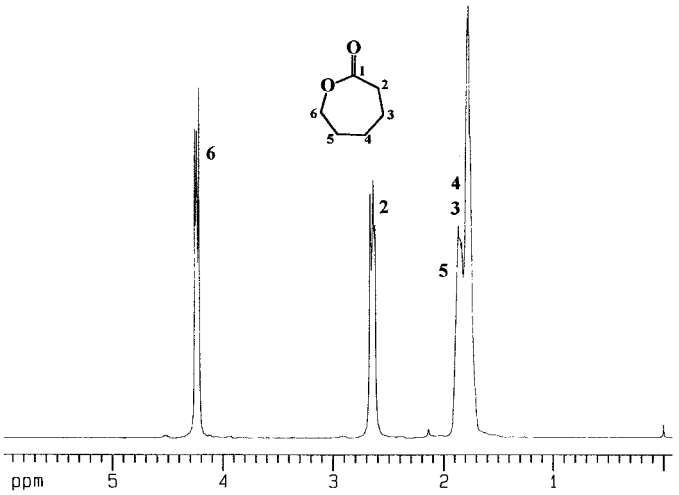

FIG. 1. The 250 MHz ^{1}H Proton NMR spectrum of ϵ-caprolactone in $CDCl_3$.

Scheme I) ($-\underline{CH_2}-O-C=O$) and methylene protons next to the carbonyl ($-\underline{CH_2}-C=O$) (labeled 2) appear as triplets at 4.25 and 2.65 ppm, respectively. The methylene protons labeled as 3 and 5 appear as a multiplet at 1.90 ppm and a broad peak for methylene protons (labeled 4) at 1.75 ppm. The ring-opened product, poly(ϵ-CL), also shows similar resonances in its ^{1}H NMR spectrum. (The proton NMR spectrum of this compound is not shown here.) The resonances are, however, shifted upfield for poly(ϵ-CL) as expected due to its linear structure. As a result of ring-opening, the methylene resonances of ϵ-CL at 4.25 and 2.65 ppm are shifted to 4.05 and 2.35 ppm in poly(ϵ-CL), respectively. The signal pattern in the spectral region of 1.5–1.9 ppm for methylene protons (labeled as 3′-5′ in Scheme I) are also changed as compared with the corresponding protons in ϵ-CL. Additionally, the end group methylene resonances ($-\underline{CH_2}-OH$) appear as a triplet at 3.75 ppm.

Ethyl glucopyranoside was prepared from D-glucose by reaction with ethanol under strong acidic conditions (see Experimental section). EGP has a complex resonance pattern in its ^{1}H NMR spectrum (Fig. 2a). Protons on the carbon C-1 in the α and β anomers appear as doublets at 4.65 and 4.15 ppm, respectively. These resonances were used to calculate the anomeric composition of the EGP. NMR integrations suggest that the ratio of the relative amount of α and β anomers in EGP was 2.1:1.0. Due to the presence of α and β anomers in the sample, two triplets appear for methyl protons at 1.15 ppm. The resonances in the 2.85–3.80 ppm are due to the remaining protons on the sugar ring.

^{1}H-NMR Spectrum of the Product: EGP-Oligo (ϵ-CL). The 250 MHz ^{1}H NMR spectrum of the product, EGP-oligo(ϵ-CL), in Fig. 2b shows the resonances due to both (1) the oligo(ϵ-CL), and (2) the ethyl glucose head group. The multiplets at 1.35, 1.56, and 2.40 ppm and the triplets at 3.35 and 4.05 ppm are representative for oligo(ϵ-CL), and the resonances for ethyl glucose head group appear as a triplet at 1.15, multiplets from 3.00–3.70 and 4.05–4.44 ppm, and a doublet at 4.65 ppm. Thus the presence of resonances due to ethyl glucopyranoside and poly(ϵ-CL) in the ^{1}H NMR spectrum of the product suggested the formation of an oligomer containing an EGP head group. On the basis of the NMR data, we will also discuss later in this section the linkage between the head group and oligo(ϵ-CL).

Reactions involving anomeric mixtures present the possibility of substrate specificity of the enzyme in preference of one or the other anomer. In fact, *Mucor miehei* and *Candida antarctica* lipase catalyzed acylation of pure ethyl β-glucopyranoside with fatty acids was reported to proceed twice as fast as that of the α anomer.[21] However,

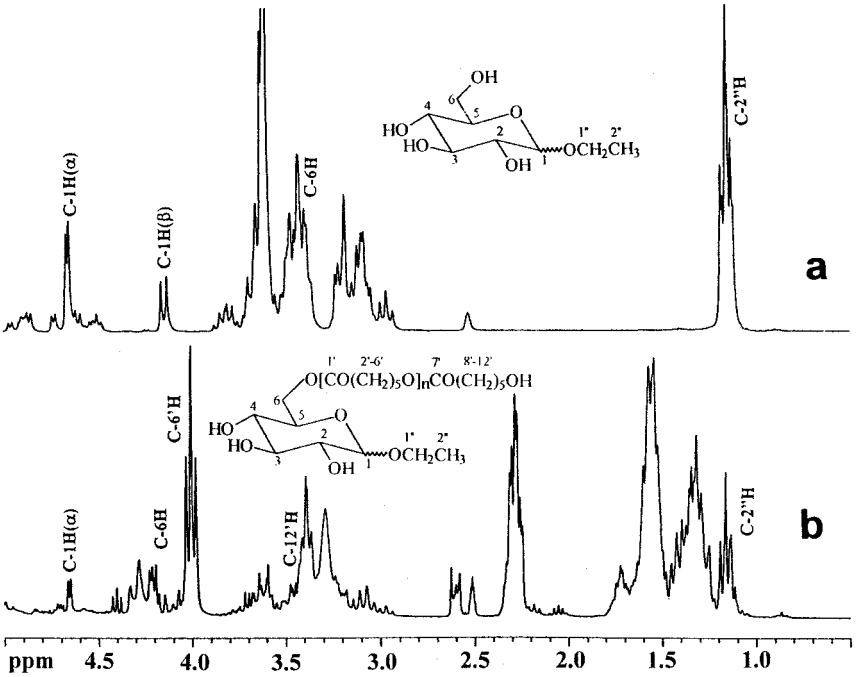

FIG. 2. The 250 MHz Proton NMR spectra of (***a***) ethyl glucopyranoside, and (***b***) the product, oligo(ϵ-CL) EGP conjugate, in DMSO-d_6.

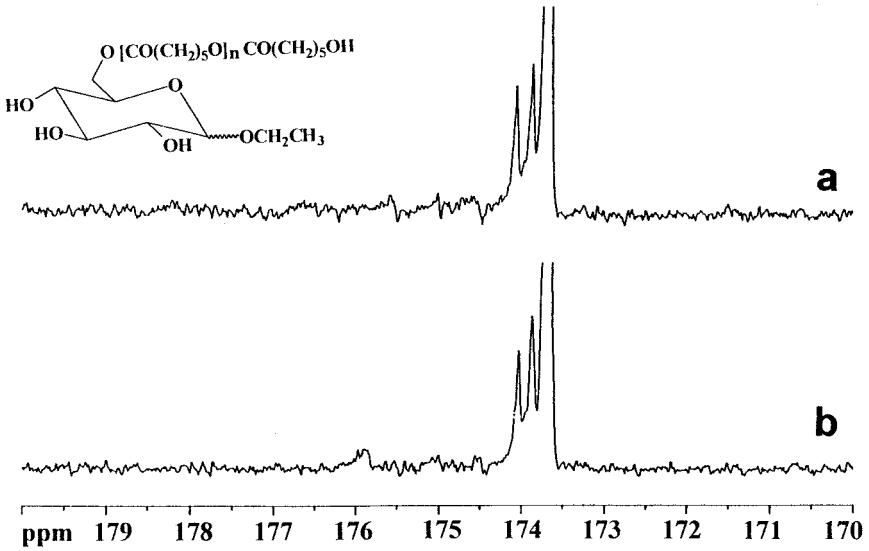

FIG. 3. Region 170–180 ppm of the 62.9 MHz ^{13}C-NMR spectra of the product, oligo(ϵ-CL) EGP conjugate, (***a***) before and (***b***) after derivatization with diazomethane in $CDCl_3$.

in the present work, porcine pancreatic lipase catalyzed ring-opening of ϵ-caprolactone with ethyl α- and β-glucopyranoside (anomeric mixture) did not indicate a preference towards either of the anomers. This observation is based on our NMR determination of the ratio of α and β anomers in the EGP-oligo(ϵ-CL). In the proton NMR spectrum (Fig. 2b) of the product, an EGP-oligo (ϵ-CL), the anomeric ratio of 2:1 was the same as in the starting material, EGP (Fig. 2a). The area of the doublet at 4.65 ppm to that at 4.15 ppm was used to estimate the anomeric ratio.

Ethyl glucopyranoside as a multifunctional initiator having four hydroxyl groups presents a challenging task in the ring-opening of ϵ-CL. These hydroxyl groups have similar reactivities and they can equally participate in the ring-opening reaction. It is difficult to distinguish various reaction sites involving primary and secondary hydroxyl groups. There is no general basis for positionally specific participation among the three secondary hydroxyl groups. In addition, distinction between the chemical reactions involving primary and secondary hydroxyls is also not easy. Therefore determination of the site(s) of reaction in EGP initiated ϵ-CL ring-opening polymerization is important. In simple cases, it is possible to identify the site of chemical linkage between EGP and oligo-ϵ-CL by comparing the chemical shifts of ethyl glucopyranosides before and after the reaction. However, as a result of overlapped resonance peaks in the proton NMR spectrum of the product, it is difficult to assign all the resonances to identify the possible links between the oligo(ϵ-CL) chain and the EGP head group. Alternatively, ^{13}C-NMR can be used for determination of the position of the link(s) due to the wide dispersion of chemical shifts in comparison with ^{1}H NMR.

^{13}C NMR Spectrum of the Product, EGP-Oligo(ϵ-CL). Figure 3a displays the region from 170 to 180 ppm of the ^{13}C-NMR spectrum of the product. The ^{13}C-NMR spectrum of the product also supported attachment of ethyl glucopyranoside to the poly(ϵ-CL) chain (full spectrum not shown). Resonances at 173.8, 64.4, 34.4, 28.7, 25.9, and 24.9 ppm due to the carbons of the poly(ϵ-CL) together with distinct low intensity peaks due to the carbons of ethyl glucopyranoside at 103.5, 99.3, 77.4, 74.5, 74.2, 74.1, 72.7, 71.4, 71.1, 70.58, 64.8, 64.5, 63.47, 16.0, and 15.8 ppm were present. The resonance ascribable to the α-methylene carbon of the hydroxyl end group of the oligo chain was observed at 62.8 ppm. The assignments of resonances in Fig. 3a suggest the absence of the carboxylic acid end group in the product. Water in lipase-catalyzed ring-opening polymerization of ϵ-CL is known to act as an initiator.[24–26] Thus in the present reaction, water may act as a competing initiator. If a fraction of the oligo(ϵ-CL) chains were initiated by water, this procedure would result in carboxyl terminal chain ends having a carbonyl signal in the region between 178 and 176 ppm.[27] We do not observe these carboxyl carbon resonances in Fig. 3a. The downfield peaks (173–174 ppm region) in Fig. 3a are due to carbonyl carbons of the oligo chain. The absence of carboxyl resonances in Fig. 3a suggests that the initiation of the oligo(ϵ-CL) chain was by EGP and not by the water.

The absence of carboxyl groups in the product was also tested by derivatization of the product with diazomethane. As a result of diazomethane derivatization, conversion of carboxyl terminal groups to their methyl ester derivative would cause a 4 ppm upfield shift in corresponding carbonyl signals. In a control experiment, oligo(CL) was prepared by the PPL-catalyzed ring-opening polymerization of ϵ-CL.[24] Terminal carboxyl functionalities of oligo(CL) were esterified with diazomethane, and its ^{13}C-NMR spectrum before (Fig. 4a) and after (Fig. 4b) derivatization were compared. The signals observed for oligo(ϵ-CL) at 177.8 and 178.1 ppm assigned to carboxylic acid C=O groups were shifted upfield by 4 ppm subsequent to esterification. Importantly, upon derivatization with diazomethane the ^{13}C-NMR spectrum of the product, oligo(ϵ-CL) EGP conjugate (Fig. 3b), did not show any changes in chemical shift of the peaks in the 173–174 ppm region. This observation confirms our earlier assignment of these peaks to carbonyl moieties. It

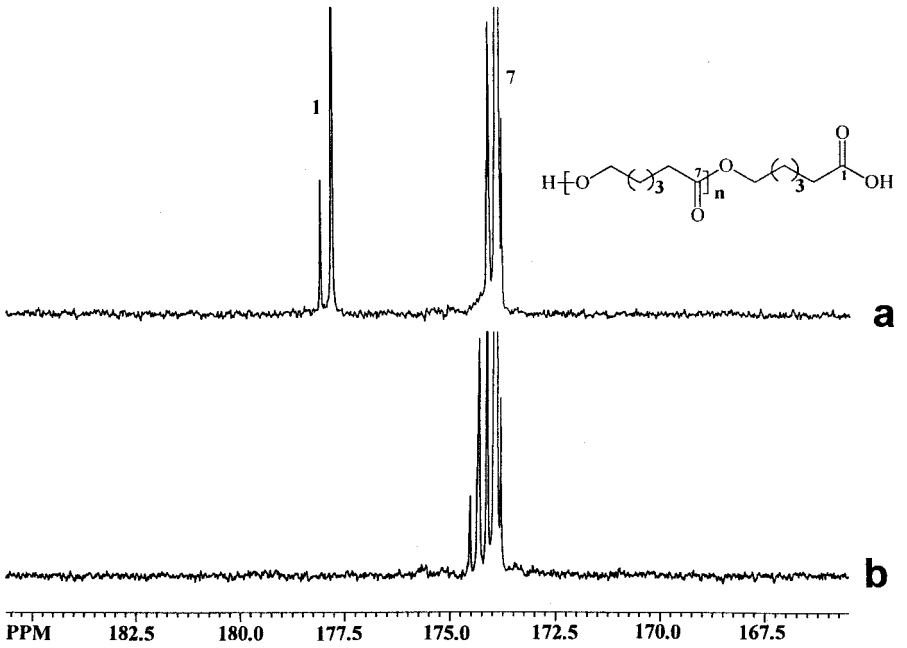

FIG. 4. Region 170–180 ppm of the 62.9 MHz ^{13}C-NMR spectra of oligo(ϵ-CL) (***a***) before and (***b***) after derivatization with diazomethane in $CDCl_3$.

means that the initiation in our reaction was caused by EGP and not by water.

To establish the link(s) between poly(ϵ-CL) side chain(s) and the EGP, we need conclusive assignments of individual carbon resonances on the EGP ring. The interpretation of the ^{13}C-NMR spectrum is complicated since the resonances from the α and β anomers of EGP and methylene carbons from the poly(ϵ-CL) side chain are crowded, especially in the 60–80 ppm region. The ^{13}C-NMR DEPT (distortionless enhancement by polarization transfer) experiment is useful in distinguishing resonances from different types of carbons.[28] With the assistance of this experiment, it is possible to edit the ^{13}C-NMR spectra showing resonances due only to methyl, methylene, methine, or quaternary carbons. The DEPT-90 experiment provides a spectrum showing the resonances only for the methine carbons. On the other hand, the DEPT-135 experiment shows positive and negative resonances to distinguish methyl, methine, and methylene resonances. The positive peaks in DEPT-135 are due to methyl and methine carbons, while negative peaks are due to methylene carbons. We used the DEPT-135 experiment to edit the complex ^{13}C NMR spectrum of the product. To assist in the assignments of resonances of EGP-Oligo(ϵ-CL), we also recorded the DEPT-135 spectrum of the starting material, EGP.

In the ^{13}C DEPT-135 spectrum of EGP, all carbohydrate signals (i.e., 60–80 ppm) were resolved (Fig. 5a). In Fig. 5, positive peaks are for resonances of –CH, $-CH_3$ types of carbons, whereas negative peaks are for $-CH_2-$ types of carbons in the molecule. In the DEPT-135 spectrum, there are no resonances for the quaternary carbons. Even though we were able to distinguish different types of carbons in EGP, we were unable to make complete assignments in spite of assistance from the published literature on these molecules.[20,21] In order to make unequivocal assignments of all carbon resonances in the mixture of α and β anomers of EGP, a two-dimensional NMR experiment on ^{13}C enriched EGP was performed. Assignments of all carbons signals in EGP were made from a ^{13}C–^{13}C COSY experiment of 99% ^{13}C enriched EGP (Fig. 6; for synthesis, see Experimental section).

Assignments for α and β Anomers. The expanded region from 55 to 85 ppm of the ^{13}C–^{13}C COSY spectrum of the ^{13}C-labeled ethyl glucopyranoside showing methylene and methine carbon resonances is shown in Fig. 6. The contour plot of the ^{13}C–^{13}C COSY experiment provides carbon–carbon connectivities. With knowledge of the assignment for one carbon, it is easy to find the connectivities for other carbons in the molecule. Peaks at 103.4 and 99.2 ppm were assigned to the anomeric carbons in β and α anomers, respectively.[21]

First we discuss the assignments of the carbons for the β anomer. The β anomeric carbon (β C-1) at 103.4 ppm shows a cross-peak to the resonances at 74.3 ppm. On the basis of this connectivity, we assign the resonance peak at 74.3 ppm to the carbon C-2 in the β anomer (β C-2). Further, the resonance at 74.3 ppm (β C-2) shows an additional cross-peak to the carbon resonance at 77.6 ppm. On the basis of connectivity, the resonance at 77.6 ppm is due to β C-3 resonance shows the connectivity to β C-4 at 71.0 ppm. The carbon C-4 at 71.0 ppm has a cross-peak to the resonance of the carbon β C-5 at 77.6 ppm. The peak for β C-5 is also connected to the peak of β C-6 at 62.0 ppm. The ethyl group was not labeled and therefore is not observed in the present setup of the experiment. In a similar way, starting from the resonance for the carbon α C-1 at 99.2 ppm, we are able to assign all the peaks for the α anomer. Assignments for the α anomer are also shown in Fig. 6.

Our data show that chemical shifts of different carbons in the EGP are sensitive to the stereochemistry at the anomeric carbon (C-1). The chemical shift difference between respective carbons in α and β anomers varied from

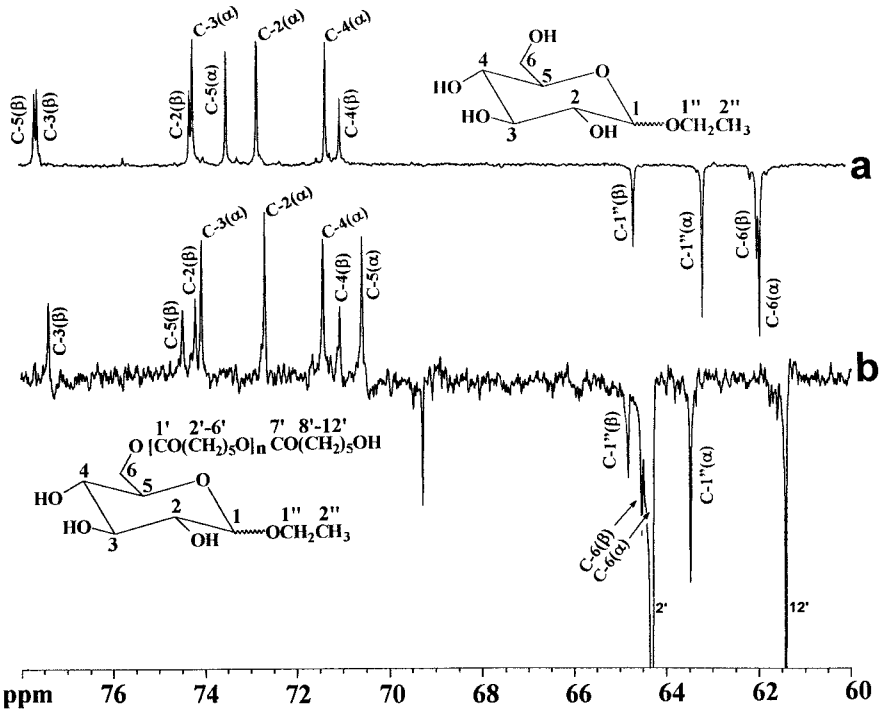

FIG. 5. Region 60–77 ppm of the 62.9 MHz DEPT-135 ^{13}C-NMR spectra of (***a***) ethyl glucopyranoside, and (***b***) the product, oligo(ϵ-CL) EGP conjugate, in DMSO-d_6. The resonance at 69.2 ppm is due to residual unreacted ϵ-caprolactone.

0.3 to 4.2 ppm (Table I). Carbon C-6 shows a chemical shift difference of 0.1 ppm, while the chemical shift difference for carbon C-3 is 3.2 ppm. The largest difference in the chemical shift was observed for the anomeric carbon itself (C-1).

^{13}C-NMR Spectrum Analysis for the Oligo(ϵ-CL)-EGP. In Fig. 5, we show the 60–80 ppm region of the ^{13}C-NMR (DEPT-135) spectrum for (**a**) ethyl glucopyranoside (EGP) and (**b**) EGP oligo(CL). The assignments and structures are also shown in Fig. 5. Comparison of the ^{13}C-NMR data for EGP and the oligomer suggests a 2.4 ppm downfield shift for carbon C-6. This difference of chemical shift is observed for both α and β anomers (Fig. 5). The peaks at 61.9 and 62.0 ppm, which are due to carbons C-6 in α and β anomers, moved downfield to 64.5 ppm and 64.8 ppm in the oligo(CL)-EGP (Fig. 5b), respectively. The resonances at 64.3 and 61.2 ppm are assigned to the caprolactone's oxymethylenes and the end group, respectively, in the oligo(ϵ-CL) chain.

Furthermore, an upfield shift of about 3.0 ppm is observed for the carbon C-5 in both α and β anomers in the oligo(ϵ-CL) EGP conjugate when compared with that of the EGP. The contributing factor for the upfield shift to these resonances for the oligomer is discussed below.

^{13}C-NMR Identification of the Site of Linkage. As discussed here and elsewhere,[21] the determination of reaction site(s) in the EGP ring is particularly difficult due to the presence of four possible reaction sites, i.e., hydroxyl groups. Three different possibilities exist: (1) random participation of different hydroxyls, leading to a

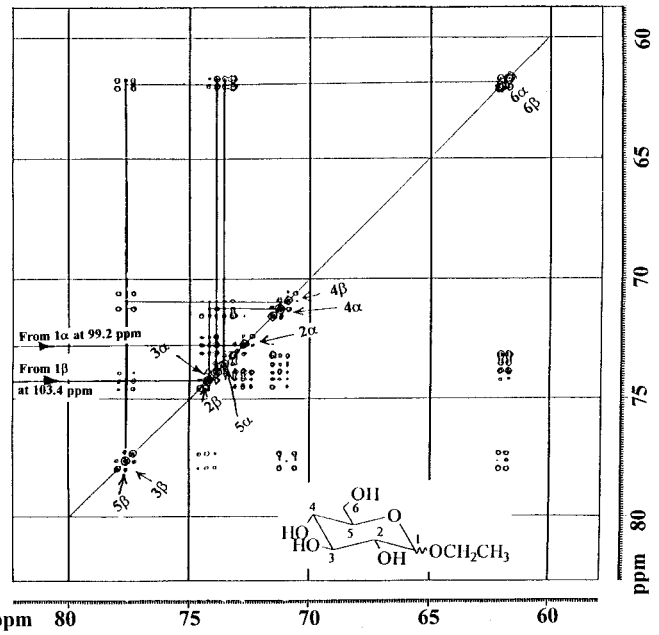

FIG. 6. Region 55–85 ppm of the 125.8 MHz ^{13}C–^{13}C NMR COSY-45 spectrum of the ^{13}C enriched ethyl glucopyranoside (^{13}C-EGP) in DMSO-d_6.

TABLE I. Chemical shift differences in the α and β anomers of ethyl glucopyranoside (DMSO-d_6).

Carbons	Chemical shifts (ppm)		Difference in the chemical shifts ($\delta\beta - \delta\alpha = \Delta$ppm)
	α Anomer ($\delta\alpha$)	β Anomer ($\delta\beta$)	
C-1	99.2	103.4	+4.2
C-2	72.8	74.3	+1.5
C-3	74.2	77.6	+3.2
C-4	71.3	71.0	−0.3
C-5	73.5	77.6	+4.1
C-6	61.9	62.0	+0.1
C-1″	63.4	64.6	+1.2
C-2″	14.6	14.8	+0.2

complex mixture; (2) equal participation of different hydroxyls, resulting in a product in which all hydroxyls take part in the ring-opening process; and (3) regioselective reaction at a specific hydroxyl position. In any of these cases, changes in chemical shifts of the participating carbons will provide important information regarding participation of the individual hydroxyls. Now we discuss the importance of the NMR data to determine the position of the possible link(s) of EGP in the lipase-catalyzed ring-opening polymerization of ϵ-CL.

The significant changes in chemical shifts for the carbon C-6 and C-5 in Fig. 5 strongly suggest the influence of the polymer link to the EGP at carbon C-6. The coupling of EGP to the polymer chain is clearly evidenced by the chemical shift change for the carbon C-6. There is a 2.5 ppm downfield shift as result of EGP linkage to the polymer chain at the C-6. This chemical shift change is possible only if the polymer is attached at the carbon C-6. Additionally, an upfield shift of 3.0 ppm for the carbon C-5 is a strong indication of the γ effect to this carbon by the ester carbonyl of the polymer chain.[29] Importantly, there is no significant influence on carbons C-2 to C-4. On the basis of these observations, our NMR data strongly suggest that the C-6 hydroxyl of EGP serves as a site for lipase-catalyzed ring-opening polymerization of ϵ-CL. There is no significant impact on the chemical shifts of other carbons of EGP as a result of lipase catalyzed ring-opening polymerization of ϵ-CL. The NMR data thus lead us to conclude that the ring-opening polymerization of ϵ-CL was a regioselective one with exclusive participation of hydroxyl group on the carbon C-6 of EGP.

CONCLUSION

We have demonstrated the applicability of ^{13}C–^{13}C COSY and DEPT-135 NMR experiments in assignments of carbon resonances in a mixture of α and β anomers of ethyl glucopyranoside and the product obtained by ring-opening polymerization of ϵ-CL initiated by EGP and catalyzed by porcine pancreatic lipase. The NMR data allowed us to investigate the regioselective initiation in the lipase-catalyzed ring-opening polymerization reaction. Analysis of the data strongly suggested the incorporation of EGP to the polymer chain during ring-opening polymerization. Among all the possibilities, NMR data identified the hydroxyl on carbon C-6 as the exclusive participant in the ring-opening polymerization of ϵ-CL. This observation allowed us to conclude that PPL-catalyzed ring-opening polymerization of ϵ-CL initiated by EGP is a highly regioselective reaction.

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