Synthesis and Characterization of Novel Block Copolymer from *trans*-4-Hydroxy-L-proline and Poly(ethylene glycol)

REN-SHEN LEE,[1] JEN-MING YANG[2]

[1] Center of General Education, Chang Gung University, Taoyuan, 333, Taiwan, Republic of China

[2] Department of Chemical Engineering, Chang Gung University, Taoyuan, 333, Taiwan, Republic of China

ABSTRACT: A series of novel ABA-type block copolymers were synthesized by polymerization of *trans*-4-hydroxy-L-proline (HyP) in the presence of various molecular weight poly(ethylene glycol)s (PEGs), a bifunctional OH-terminated PEG using stannous octoate as catalyst. The optimal reaction conditions for the synthesis of the copolymers were obtained with 5 wt % stannous octoate at 140°C under vacuum (20 mmHg) for 24 h. The synthesized copolymers were characterized by IR spectroohotometry, proton nuclear magnetic resonance, differential scanning calorimetry, and Ubbelohde viscometer. The glass transition temperature (T_g) of the copolymers shifted to significantly higher temperature with increasing the number average degree of polymerization and HyP/PEO molar ratio. In contrast, the melting temperature (T_m) decreased with increasing the HyP/PEO molar ratio. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 1581–1587, 2001

Key words: poly(ethylene glycol); pseudohydroxyproline; poly(HyP-*co*-PEG)

INTRODUCTION

Poly(ethylene glycol)s (PEGs) are an important class of polymers that have found many applications in a variety of fields, and, recently, there has been special interest for their use in medicine, biology, and biomedical science. The significant properties of PEG include ease of chemical modification, biocompatibility, lack of antigenicity and immunogenicity, a wide range of solubility, and complexing ability with metal ions.[1,2]

Block copolymers of PEG with lactide,[3–12] ε-caprolactone,[13] 1,4-dioxan-2-one,[14] and trimethylene carbonate[15] have been reported by a number of investigators. These block copolymers are important polymeric biomaterials with controllable biodegradation rates. Also, incorporation of amino acids into the backbone of PEG has previously been described for use as drug carriers. ABA- or AB-type block copolymers composed of a poly(amino acid) as the hydrophobic A part and PEG as the hydrophilic B part are obtained via polymerization of *N*-carboxy anhydrides of phenylalanine,[16] γ-benzyl-L-glutamate,[17] *N*-(benzyloxycarbonyl)-L-lysine,[18] β-benzyl-L-aspartate,[19] or proline[20] initiated by primary amino groups on the termini of the PEG chain. Since these amphiphilic block copolymers form polymeric micelles or core–shell-type nanoparticles, they are useful for carrying hydrophobic drugs in drug-delivery systems.

Although PEGs have been studied intensively as biomaterials, one of the shortcomings of these linear polymers is that there are only two reactive groups (hydroxyl groups at both chain ends) and

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no pendant functional groups on the backbone molecules which could be used for covalently bonding of either drugs or biological active agents. Consequently, alternating copolymers of PEG with L-lysine[21] and L-aspartic acid[22,23] have been prepared to improve low reactive groups of PEG. The resulting copoly(PEG–amino acid)s have multiple pendant functional groups with predetermined intervals along the polymer backbone chain.

In this work, an ABA-type block of poly(*trans*-4-hydroxy-L-proline-*co*-PEG) having pendant amine groups was synthesized by the melt polycondensation reaction from *trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline methyl ester and low molecular weight PEG using stannous octoate as a catalyst. These new copolymers were identified by ^{1}H-NMR, IR, and an Ubbelohde viscometer, and their thermal properties were also examined.

EXPERIMENTAL

Materials

trans-4-Hydroxy-L-proline, benzyloxychloroformate, thionyl chloride, formic acid, titanium isopropoxide, alumina isopropoxide, zinc chloride, cesium chloride, and palladium (10%) on activated carbon and poly(ethylene glycol)s of various molecular weights (PEG 600, 1000, 2000) were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Stannous octoate was purchased from the Strem Chemical Co. (Newburyport, MA). Organic solvent (e.g., tetrahydrofuran, methanol, chloroform, *N,N*-dimethylformamide, and ethylacetate) were HPLC grade and inorganic compounds (e.g., sodium sulfate and sodium bicarbonate) were reagent grade.

Characterization

Melting-point determinations were made on a Buchi 535 melting-point apparatus, Infrared spectra were measured on a JASCO IR Report-100 infrared spectrophotometer. Samples were either neat onto NaCl plates or pressed into KBr pellets. ^{1}H-NMR spectra were recorded at 500 MHz (Brucker WB/DMX-500 spectrometer), using tetramethylsilane as an internal standard. Elemental analyses were run on a Perkin–Elmer Model 2400 C, H, N analyzer. The inherent viscosities were measured with an Ubbelohde viscometer at 30°C.

Thermal analysis of the polymer was performed on a DuPont 9900 system that consisted of a DSC differential scanning calorimeter. The heating rate was 20°C/min. Glass-transition temperatures (T_g) were read at the middle of the change in the heat capacity and were taken from the second heating scan after quick cooling.

Synthesis of Copolymer

trans-4-Hydroxy-*N*-benzyloxycarbonyl-L-proline methyl esters (N-Z-HyP) **1** were prepared according to the process in our previous study.[24] Predetermined amounts of N-Z-HyP and PEG of various molecular weights were introduced into a flask. The procedure of polymerization was comparable to that of the homopolymerization of N-Z-HyP. The catalyst stannous octoate was then added. The reaction was carried out under a vacuum (20 mmHg) at 140°C for 2 h. The crude polymer was dissolved in tetrahydrofuran and then precipitated into *n*-hexane with stirring. After purification, a white fine powder copolymer **3** was obtained.

Deprotection of Amino Protecting Group of the New Copolymer

A 10 wt % palladium-on-charcoal catalyst (2 g) was added to a solution of copolymer **3** (0.6 g) in *N,N*-dimethylformamide (8 mL). With vigorous stirring, 98% formic acid (28 mL) was slowly added to the mixture. At the beginning, the evolution of hydrogen was vigorous and ceased after about 1 h. Stirring was continued at room temperature for 14 h and then the palladium catalyst was removed by filtration and washed with 1*N* HCl (40 mL). The washing was combined with the filtrate. The combined solution was concentrated to a total volume of 10 mL by partial evaporation under reduced pressure. The concentrated solution was then mixed with 1*N* HCl (20 mL) to complete replacement of the formate salt by hydrochloride acid. Finally, the acidic polymer solution was poured into acetone to precipitate the polymer as colorless powder **4**.

RESULTS AND DISCUSSION

Synthesis of the Protected Poly(*trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline-*co*-PEG) Copolymers 3

Various PHyP/poly(ethylene glycol) (PEG) copolymers were obtained from the copolymerization of
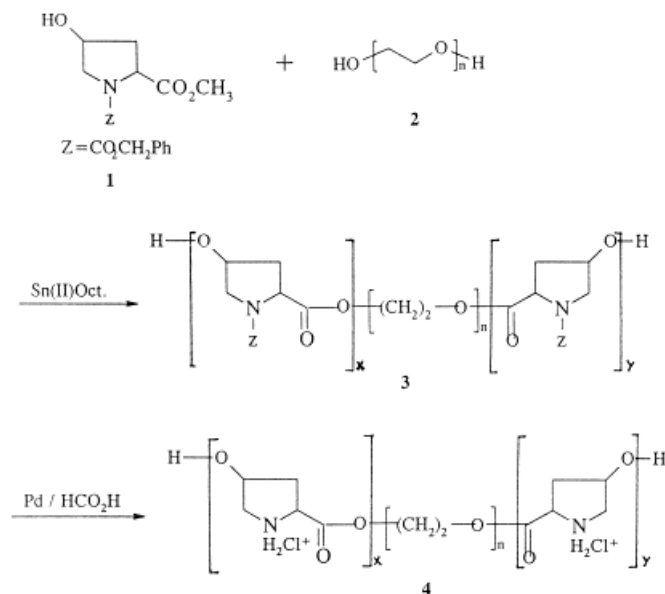
Scheme 1   Synthesis of poly(N-Z-HyP-co-PEG) **3** and its deprotection **4**.

N-Z-HyP **1** in the presence of PEG and small amounts of stannous octoate (Scheme 1). The lengths of the PHyP block in the block copolymers were controlled by changing the amount of *trans*-4-hydroxy-L-proline. To obtain the optimal reaction condition for the preparation of the copolymer, several parameters (i.e., types and amounts of acid catalysts and molecular weight of PEG) were examined in this study. The effect of the type of acid catalyst on the polycondensation of N-Z-HyP **1** with PEG 600 was carried out under a vacuum (20 mmHg) at 140°C for 24 h and the data are summarized in Table I. The type of acidic catalyst appeared to significantly affect the yield (20–88%) and inherent viscosity of the resulting copolymers (η_{inh} = 0.31–0.86). The stannous octoate produced the highest yield (88 %) and mod-

erate inherent viscosity (η_{inh} = 0.61). The titanium isopropoxide produced the highest inherent viscosity (η_{inh}= 0.86), but the yield was low.

The effect of the amount of the acid catalyst on the synthesis of the copolymer was examined by using stannous octoate with PEG 600 for 24 h. Table II shows the results of the yield and inherent viscosity (η_{inh}) of the copolymers obtained. The highest yield was achieved when 5 wt % of the acid catalyst was used under a vacuum (20 mmHg). The η_{inh} increased with an increasing amount of stannous octoate. Under a nitrogen atmosphere, the highest η_{inh} and a moderate yield was obtained. Under a nitrogen atmosphere

Table I   Preparation of Polymer 3 by Polycondensation Reaction of N-Z-HyP 1 and PEG 600 with Various Acid Catalysts

Run	Catalyst	Yield (%)	η_{inh} (dL/g)[a]
1	Sn(II)Oct.	88	0.61
2	Ti[OCH(CH$_3$)$_2$]$_4$	23	0.86
3	Al[OCH(CH$_3$)$_2$]$_3$	84	0.52
4	ZnCl$_2$	66	0.31
5	CsCl	20	0.43

The reaction was performed by using 5 wt % catalyst at 140°C for 24 h under a vacuum.
[a] Measured at a concentration of 0.1 g/dL in CHCl$_3$ at 30°C.

Table II   Effect of the Amount of Sn(II)Oct. Catalyst on the η_{inh} and Yield on Polymer 3 Prepared by Polycondensation of N-Z-HyP and PEG 600

Run	Sn(II)Oct. (wt %)	Yield (%)	η_{inh} (dL/g)[a]
1	1	85	0.31
2	3	77	0.41
3	5	88	0.61
4	5[b]	61	0.73
5	5[c]	94	0.41

Sn(II)Oct.: stannous octoate.
[a] Measured at a concentration of 0.1 g/dL in CHCl$_3$ at 30°C.
[b] The reaction was performed under a nitrogen atmosphere.
[c] The reaction was performed under a nitrogen atmosphere for 12 h and a vacuum (20 mmHg) for 12 h.

Table III   Molecular Weight and Thermal Properties of Various Polymers 3 Prepared with Different HyP/PEO Feed Ratios

Sample	Molecular Weight of PEO	HyP/PEO in Feed[a]	HyP/PEO in Product[b]	M_n[c]	T_m (°C)	T_g (°C)
A	600	20	13.8	4000		15
B	1000	20	16.4	5000		21
C	2000	1	0.95	2200	49	
D	2000	2	1.6	2400	44	
E	2000	5	4.5	3100	36	−37
F	2000	10	9.1	4200		−17
G	2000	20	16.7	6100		−0.3
H	2000	40	34.5	10,500		33

[a] Molar ratio of HyP over PEG.
[b] Determined by ^{1}H-NMR spectroscopy.
[c] M_n of sample = M_n (PEO) + 247 × HyP/PEO in product + 18.

for 12 h and a vacuum (20 mmHg) for 12 h, the highest yield was obtained. But the η_{inh} was low. So, the optimal reaction condition for the copolymerization is at 14°C, 24 h, under a vacuum, using 5 wt % of stannous octoate as a catalyst.

The effect of the molecular weight of PEG on the M_n of the copolymer **3** was also examined using 5 wt % of the stannous octoate catalyst for 24 h (Table III). The HyP/PEO molar ratio in the feed ranged from 1 to 40. The values of the HyP/PEO ratio on the copolymers were evaluated from the ^{1}H-NMR spectra.

^{1}H-NMR

^{1}H-NMR spectra of PHyP/PEO block copolymers were used to determine the HyP/PEO ratio from the integration ratio of resonance due to PEO blocks at 3.65 ppm (—O—CH$_2$—CH$_2$) and to PHyP blocks at 7.33 ppm (phenyl). After purification, all the resulting copolymers exhibited a HyP/PEO molar ratio close to those of corresponding feeds (Table III). The higher the HyP/PEO ratio and molecular weight of PEG in the feed, the higher the M_n.

Thermal Analysis

Thermal characteristics of various copolymers are also presented in Table III. The T_m of the parent PEG polymers increased with their molecular weight: 21°C for PEG 600, 42°C for PEG 1000, and 50°C for PEG 2000. When PHyP attached to PEO with a PHyP/PEO feed ratio lower than 5, the melting temperature of the copolymers decreased. For samples **C**, **D**, and **E**, the T_m values

were 49, 44, and 36°C, respectively. When the PHyP/PEO feed ratio was higher than 5, T_m's were not detected (samples **F**, **G**, and **H**). The T_g of various copolymers increased with the PHyP/PEO feed ratio. It was found that the T_g was located at about −60°C for PEG 2000 and the T_g increased from −37 to 33°C for samples **E**, **F**, **G**, and **H**. This is because the HyP component is more rigid than are the PEG segments.

Deprotection of the *N*-Protected Poly(*trans*-4-hydroxy-L-proline-*co*-PEG)

The benzyloxycarbonyl (CBz) protecting group of copolymer **3** is usually removed by solvolysis with HBr/HOAc. However, the strong acid could lead to chain scissions of the polymer due to acid-catalyzed ester hydrolysis. To eliminate this problem of acid-catalyzed chain fragmentation, we adopted a different deprotection method, that is, catalytic transfer hydrogenation. This method is generally used in peptide chemistry to yield free amine without degrading the polymer chains. Formic acid was used as an effective hydrogen donor under mild conditions. Palladium over activated carbon (10 wt %) was used as the catalyst for the hydrogenolysis of the aromatic CBz protecting group. The T_g of the protected copolymer of sample **H** decreased from 33 to 15°C upon this deprotection reaction.

Structure Characterization

The infrared (IR) spectrum of parent PEG 2000 **2** [Fig. 1(A)] shows the characteristic absorption peaks at 3200–3600 (O—H) and 1120 cm^{-1} (C—
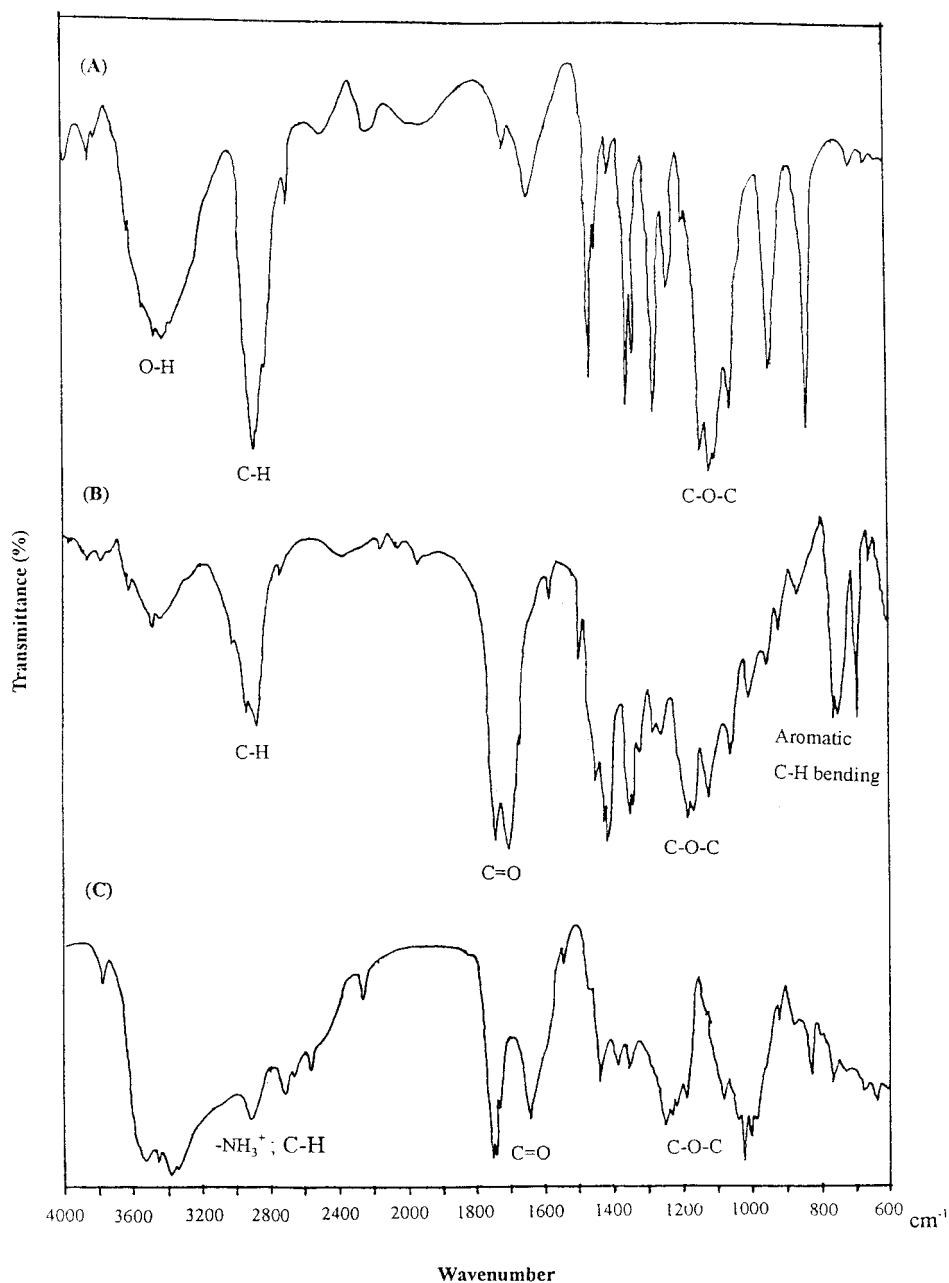
Figure 1 IR spectrum of (A) PEG 2000 **2**, (B) *N*-CBz protected sample, and (C) deprotected sample **H**.

O). The spectra of the protected copolymer of sample **H** [Fig. 1(B)] and deprotected sample **H** [Fig. 1(C)] exhibit strong ester carbonyl bands at 1720–1740 and 1742 cm^{-1}, respectively. The most distinctive features of the deprotected sample **H** [Fig. 1(C)] were the absence of the aromatic C—H (out-of-plane bending) absorption at 699 and 743 cm^{-1} from the CBz protecting group and the presence of a broad ammonium band (—NH$_3^+$) at 2600–3100 cm^{-1} (superimposed with aliphatic

C—H stretching). This indicates the successful removal of the CBz group and the formation of the pendant amine group.

The ^{1}H-NMR spectra of (A) the *N*-CBz protected sample **H** and (B) the deprotected sample **H** are shown in Figure 2 and are consistent with the IR data. Particularly, the disappearance of the signals at both 7.3 ppm (due to the protons of aromatic rings of the CBz protecting group) and at 5.1 ppm (due to benzyl methylene proton) in
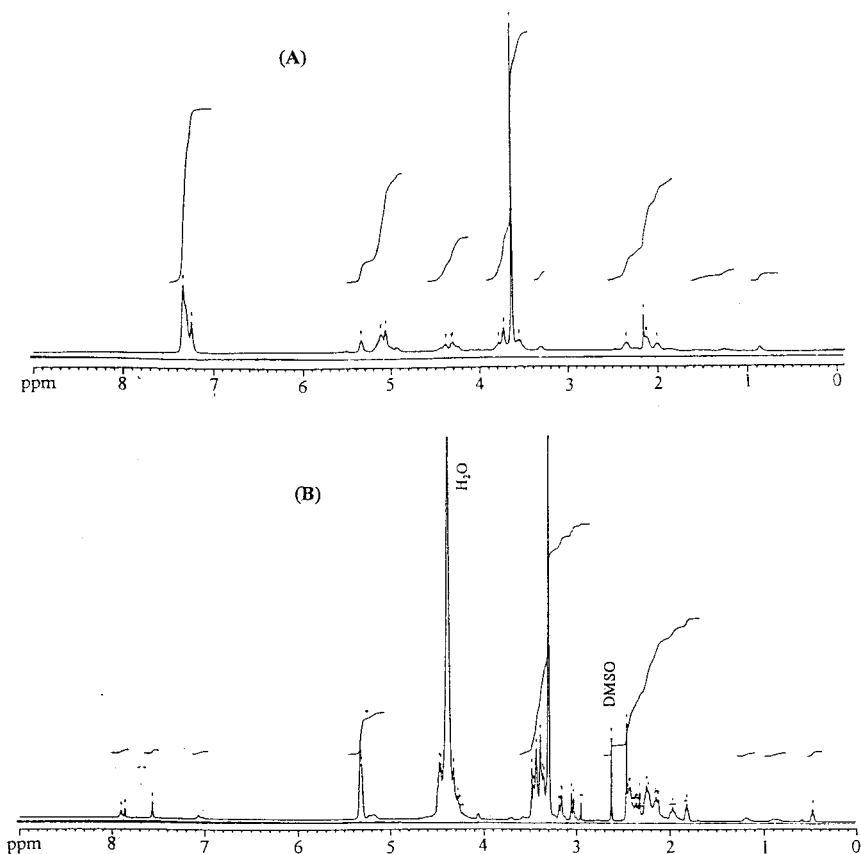
Figure 2 ^{1}H-NMR spectra of (A) *N*-CBz protected sample **H** in CDCl$_3$ and (B) deprotected sample **H** in D$_2$O/DMSO-d_6.

the deprotected sample **H** is a clear indication of the successful deprotection reaction. Also, the methylene protons of the PEG segment of the *N*-protected sample **H** exhibit peaks at around 3.6 ppm that are consistent with the protected data of other PEG-based polymers.

CONCLUSIONS

New biodegradable poly(ether ester)s having pendant amine functional groups were synthesized by melt polycondensation of N-Z-HyP and low molecular weight PEG using acid catalysts. The resulting copolymers contained a reactive amine functional group on the ring of proline residue of the copolymer. The physical properties can be controlled by choosing the appropriate PEG molecular weight and the feed ratio of PHyP and PEO.

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