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SYNTHESIS OF POLY(γ-*tert*-BUTYL L-GLUTAMATE): INFLUENCE OF POLYMERIZATION CONDITIONS

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ABSTRACT

This work presents the synthesis and characterization of poly(γ-tert-butyl L-glutamate) (PtBuLG) via a living ring-opening polymerization procedure of γ-tert-butyl L-glutamate N-carboxyanhydride (tBuLG-NCA). The reaction conditions were investigated to optimize the polymerization yield and molecular weight polydispersity. The synthesized PtBuLG was characterized using nuclear magnetic resonance spectroscopy (NMR), gel permeation chromatography (GPC) and attenuated total reflection-Fourier transform infrared (ATR FT-IR. Finally, hydrolysis of PtBuLG resulted in poly(L-glutamic acid) (PLGA).

***Keywords*:** polypeptide, *N*-carboxyanhydride, *tert*-butyl glutamate.

1. Introduction

Polypeptides have a unique position among synthetic polymers, as they have the ability to arrange possess in a long range chain order (Block, 1983). Polypeptides can have the ability of anion binding, electron and ion conduction and transfer (Hol, 1985; Quiocho, Sack, & Vyas, 1987). Living initiating ring-opening polymerization of *N*-carboxyanhydride (NCA) monomers was first introduced by Deming in 1997 (Deming, 1997). Since then, a variety of synthetic polypeptides with designable side-groups have been designed and prepared mainly for bio-related applications (Carlsen & Lecommandoux, 2009; Deng et al., 2014; Dreher et al., 2008). Up to now, polypeptides and their derivatives synthesized by ring-opening polymerization of NCAs still receive great attention not only because they are bio-compatible materials but also due to their adoptable multitude secondary structures in imitating the functions of proteins. Recently, the ability of polypeptides to form complexation with polyion has also been exploited for fabrication of self-healing materials (Sun et al., 2017).

Poly(L-glutamic acid) (PLGA) is a water soluble, anionic, biodegradable and non-toxic homo-polyamino acid. PLGA and its derivatives are thus attractive for various industrial fields, such as cosmetics, medicine, food and water treatments. PLGA can generally be synthesized by microorganisms or via synthetic pathways such as ring-opening polymerization of a glutamate NCA monomer bearing a carboxylic acid protecting group such as the piperonyl group (Shih, Van, & Shen, 2004).

In this study, poly(γ-*tert*-butyl L-glutamate) (PtBuLG) was synthesized. The primary amine-initiated NCA polymerization at low temperatures (below room temperature) approach was chosen, owing to the simplicity and the "living" character of the procedure (Vayaboury, Giani, Cottet, Deratani, & Schué, 2004). The challenge of the ring-opening polymerization of tBuLG-NCA is low yield (Cornille, Copier, Senet, & Robin, 2002; Miyazawa, 1960; Wilder & Mobashery, 1992). Therefore, the reaction conditions were investigated. As the *tert*-butyl moiety is an acid-labile protecting group for the carboxylic acid group, PLGA could be obtained via hydrolysis of PtBuLG.

2. Experiment

2.1. Materials

N-(*tert*-butoxycarbonyl)-L-glutamic acid γ-*tert*-butyl ester (Boc-Glu(OtBu)-OH) (Fluka, 99%), α-pinene (Aldrich, 98%), triphosgene (Aldrich, 98%), *n*-hexylamine (Fluka, 98%) and trifluoroacetic acid (TFA) (Merck, 99%) were used as received. All solvents used for synthesis were dried and distilled before use according to standard procedures.

2.2. Characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in deuterated solvents with TMS as an internal reference, on a Bruker Avance 300 at 300 MHz. Attenuated total reflectance (ATR) FT-IR spectra were collected as the average of 128 scans with a resolution of 4 cm^{-1} on a FT-IR Tensor 27 spectrometer equipped with a Pike MIRacle ATR accessory with a diamond/ZnSe element. Size exclusion chromatography (SEC) measurements were performed on a Polymer PL-GPC 50 gel permeation chromatograph system equipped with an RI detector, with chloroform as the eluent at a flow rate of 1.0 mL/min. Molecular weight and molecular weight distribution were calculated with reference to polystyrene standards.

2.3. Synthesis of γ-tert-butyl L-glutamate N-carboxyanhydrides (tBuLG-NCA)

All of the reaction, washing and purification steps were carried out under dry nitrogen. A mixture of 6.0 g (19.8 mmol) of Boc-Glu(OtBu)-OH, 7 mL (6 g, 44 mmol) of α-pinene and ethyl acetate was heated to 65 ^{o}C and stirred for 20-30 min. After an addition of 4.0 g of triphosgene (13.5 mmol), the reaction mixture was stirred at the same temperature for 24 h. The completion of the reaction was determined by ^{1}H-NMR (complete disappearance of δ 5.19-5.27 (d, NH) and 4.20-4.36 (α-CH) ppm). After evaporation of the solvent, the product was recrystallized three times from 40 mL of CH_2Cl_2/*n*-hexane (1:3, v/v). The obtained white product was dried under vacuum and stored under nitrogen at -18 ^{o}C.

2.4. Synthesis of poly(γ-tert-butyl L-glutamate) (PtBuLG)

PtBuLG was synthesized by polymerization of tBuLG-NCA at -10–10 ^{o}C using a primary amine initiator. Typical procedure: In a round-bottom flask capped with a rubber septum and under dry nitrogen, tBuLG-NCA was dissolved in chloroform. The reaction

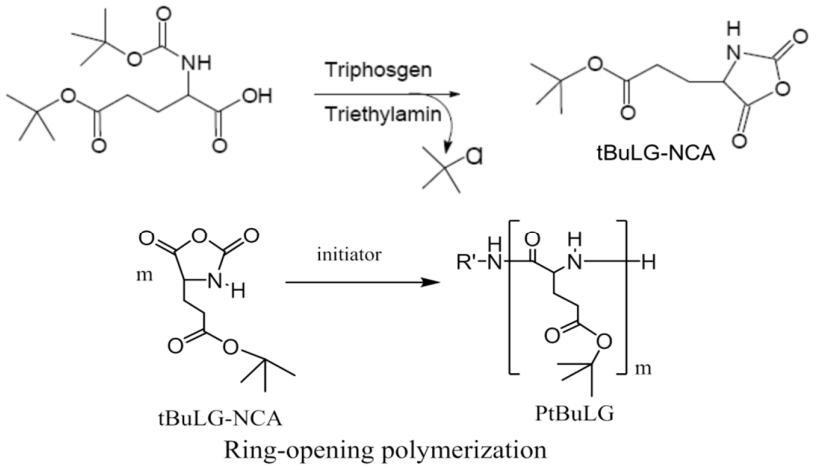
mixture was cooled to the designed temperature and a volume of *n*-hexylamine was injected via a syringe. The reaction mixture was stirred at the appropriate reaction temperature for a week. Then, the reaction solution was poured into a large amount of ethanol and chloroform was removed by rotary evaporation. The polymer precipitate was collected by filtration, washed extensively with cold ethanol, and dried at 50 ^{o}C under vacuum.

2.5. Synthesis of poly(L-glutamic acid) (PLGA)

PtBuLG was dissolved in TFA/dichloromethane (1/5, v/v) and then stirred for 1 hour. The resulting PLGA was precipitated into methanol, washed several times with methanol and dried under vacuum at 50 ^{o}C.

3. Results and discussion

The synthetic route to PtBuLG is described in Scheme 1.



Scheme 1. *Synthesis of PtBuLG*

3.1. Synthesis of monomers

tBuLG-NCA was prepared employing the method described previously (Wilder & Mobashery, 1992). A modification based on another procedure (Cornille et al., 2002) was applied, in which α-pinene was used instead of triethylamine as a HCl capturing agent. The monomer was obtained with a reasonable yields and high purity, as determined by the ^{1}H NMR result. The ^{1}H NMR spectrum of tBuLG-NCA with all characteristic peaks well assigned, is shown in Figure 1. The signal corresponding to *tert*-butyl protons is at 1.45 ppm, whereas the characteristic signal attributed to the ring amide proton is observed at 6.46 ppm.

The Fourier transform-infrared (FT-IR) spectrum of tBuLG-NCA shows characteristic absorption bands of a NCA structure, including the bands at 3350 and 1728 cm^{-1} assigned to the NH and ester C=O stretch vibration, respectively (Figure 2). The bands at 1859 and 1790 cm^{-1} are characteristic of the asymmetric and symmetric anhydride C=O stretch vibrations.

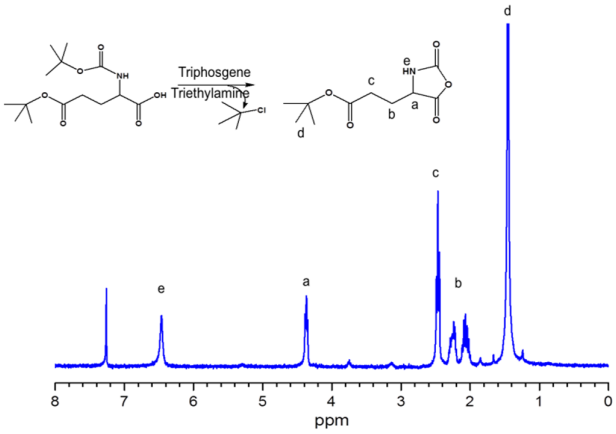


Figure 1. *^{1}HNMR spectrum of tBuLG-NCA*

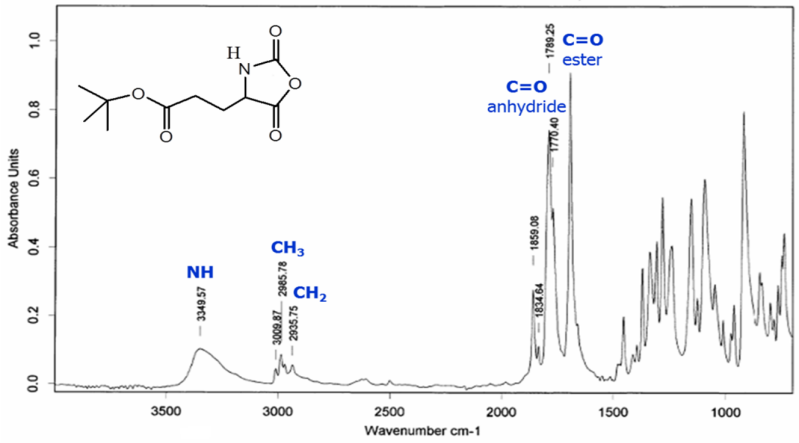


Figure 2. *FT-IR spectrum of tBuLG-NCA*

3.2. Synthesis of PtBuLG

Then, PtBuLG was synthesized through ring-opening polymerization of tBuLG-NCA. Unreacted monomer was eliminated via polymer precipitation. A typical ^{1}H NMR spectrum of PtBuLG in $CDCl_3$ is presented in Figure 3, with all characteristic peaks well assigned. The signal of the amide proton was observed at 8.2 ppm, while the signal attributed to the tert-butyl protons appeared at 1.45 ppm.

A typical ATR FT-IR spectrum of PtBuLG is shown in Figure 4. The FT-IR spectrum of PtBuLG shows characteristic absorption bands of a polyamide structure, including the bands at 1723, 1650, 1544, 1367 cm^{-1} assigned to the ester C=O stretch, amide I, amide II and *tert*-butyl C-H vibrations, respectively. The polymer conformation is completely α-helical, as identified by the amide I absorption band at 1650 cm-1 and amide II absorption band at 1544 cm^{-1}(Miyazawa, 1960). Varying the reaction conditions (temperature and monomer concentration) in the synthesis of PtBuLGs with degrees of polymerization (DPs) in the range of 14-70 did not noticeably affect the polymer

conformation. However, the polymerization conditions were found to considerably affect the molecular weight polydispersity (Đ) as well as polymerization rate, and hence the yield. Therefore, the monomer to initiator molar ratio was fixed at 60, while the monomer concentration and reaction temperature were varied. The results are summarized in Table 1.

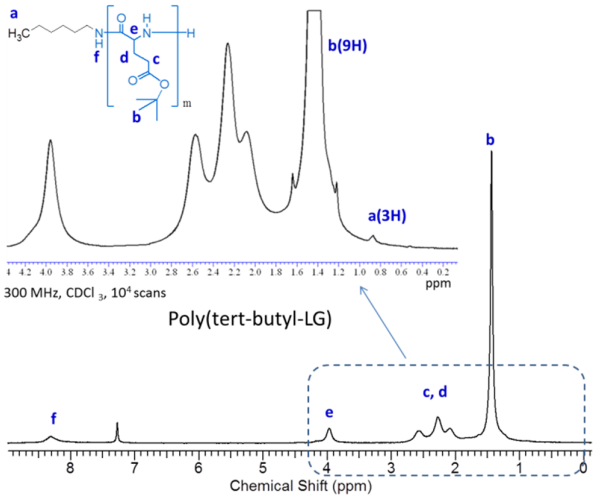


Figure 3. *^{1}H NMR spectrum of PtBuLG. Inset: zoom-in of the spectrum in the range of 0–4 ppm*

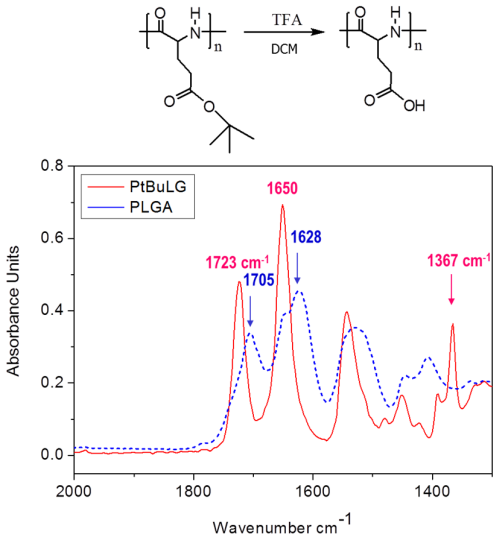


Figure 4. *FT-IR spectrum of PtBuLG and PLGA obtained by hydrolysis of PtBuLG*

As a result of the low polymerization temperature as well as the relatively low reactivity of the tBuLG-NCA monomer, the reaction occurred fairly slowly. In order to improve the reaction yield, the monomer concentration [M] was first investigated while temperature was fixed at 0 ºC. As shown in Table 1, the reaction yield is optimal (82%) at [M] of 0.08 g mL^{-1} (Entry 3). The molecular weight polydispersity (polydispersity index,

Đ) was 1.25, which is still fairly high. Higher monomer concentrations led to higher yields, on account of higher reaction rates. However, a too high concentration could increase the possibility of polypeptide chain aggregation and thereby lead to a reverse effect on the reaction rate. Next, the temperature was further investigated. At higher temperature of 10 ^oC, a higher yield of 93% was obtained, but the molecular weight polydispersity increased to 1.31 (Entry 5). On the other hand, the polydispersity was much improved at lower temperature of -10 ^oC (Đ ~1.13-1.14, Entry 6-7). The optimal condition was found to be -10 ^oC and [M] = 0.1 g mL^{-1}, resulting in PtBuLG with Đ = 1.14 and a reasonable yield of 80%.

Table 1. *Polymerization yield after 7 days*

Entry	Temperature (^oC)	[M] (10^{-2} g mL^{-1})	Yield (%)	$M_{n,\ GPC}$	Đ
1	0	6.5	50	5500	1.19
2	0	7.5	70	7700	1.20
3	0	8	82	9100	1.25
4	0	9	71	7900	1.22
5	10	8	93	10000	1.31
6	-10	8	65	7100	1.13
7	-10	10	80	9000	1.14

Finally, PLGA was obtained via hydrolysis of PtBuLG in a TGA/dichloromethane mixture. The FT-IR of the obtained PLGA is shown in Figure 4. Total cleavage of the *tert*-butyl groups is confirmed by the disappearance of the absorption band at 1367 cm^{-1}. The secondary structure of the obtained PLGA was indicated by the IR absorption positions of the amide I and amide II characteristic of polyglutamates (Nguyen, Ardana, Vorenkamp, ten Brinke, & Schouten, 2010). As shown in Figure 4, the obtained PLGA shows two IR amide I bands at 1648 and 1628 cm^{-1}, assigned to the α-helix and β-sheet structures, respectively (Nguyen et al., 2010). This indicates that the PLGA exhibited in both α-helical and β-sheet conformations. The presence of β-sheet structure can be beneficial for the formation of supramolecular structures potential for the fabrication of self-healing materials (Sun et al., 2017).

4. Conclusion

In conclusion, α-helical poly(*tert*-butyl L-glutamate) was suscessfully synthesized. Optimal reaction conditions were found to result in polymer with low molecular weight polydispersity and quite high yield. Poly(L-glutamic acid) exhibiting in both α-helix and β-sheet conformations was obtained via hydrolysis of the *tert*-butyl side groups.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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TỔNG HỢP POLY(γ-*tert*-BUTYL L-GLUTAMAT): ẢNH HƯỞNG CỦA ĐIỀU KIỆN POLYMER HÓA

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TÓM TẮT

Chúng tôi nghiên cứu tổng hợp và đánh giá poly(γ-tert-butyl L-glutamat) (PtBuLG) bằng quá trình polymer hóa mở vòng cho γ-tert-butyl L-glutamat N-carboxyanhydrid (tBuLG-NCA). Điều kiện phản ứng được khảo sát nhằm tối ưu hóa hiệu suất và độ đa phân tán khối lượng phân tử của polymer. PtBuLG được đánh giá bằng các phương pháp cộng hưởng từ hạt nhân, sắc kí gel và phổ hồng ngoại. Cuối cùng, thủy giải PtBuLG thu được poly(L-glutamic acid) (PLGA).

Từ khóa: polypeptid, *N*-carboxyanhydrid, *tert*-butyl glutamat.