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Synthesis of Heterotelechelic Poly(ethylene glycol)-*block*-poly(succinimide) Possessing Both Acetal and *Tert*-Butoxycarbonyl-amino Terminals with Narrow Molecular Weight Distribution

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Summary: A novel heterotelechelic linear block copolymer of poly(ethylene glycol) (PEG) and poly(succinimide) (PSI) possessing both acetal and *tert*-butoxycarbonyl-amino (Boc-NH) terminals (Acetal-PEG-*b*-PSI-NH-Boc) with a narrow molecular weight distribution (MWD) was successfully prepared by the nucleophilic attack of triethylamine (TEA) to the poly(β-benzyl L-aspartate) (PBLA) segment of Acetal-PEG-*b*-PBLA-NH-Boc. Acetal-PEG-*b*-PBLA-NH-Boc with MWD of 1.07 was prepared by living anionic ring-opening polymerization of β-benzyl L-aspartate *N*-carboxy-anhydride with α-acetal-ω-amino PEG as a macroinitiator, followed by Boc protection. The subsequent conversion of PBLA segment to PSI was successfully carried out by reacting with the catalytic amount of TEA. The characterization by ^{1}H NMR, GPC and IR demonstrates that the formation of poly(succinimide) proceeded completely without any remarkable side reactions. Acetal-PEG-*b*-PSI-NH-Boc thus obtained may have a potential utility as a targetable drug carrier in the field of drug delivery system.

Keywords: aspartic acid; diblock copolymers; PEG; succinimide; telechelics

Introduction

Block copolymers, which are composed of two or more covalently-linked polymers with different physicochemical properties, have been attracting significant interest in nanoscience and nanotechnologies in recent decades from both theoretical and practical aspects,[1] because amphiphilic block copolymers in a selective solvent of one block provides various morphologies in the submicron range. Particularly, in response to a great demand for novel drug and gene delivery systems, block copolymers and their assemblies in aqueous milieu have been recognized as promising formulations in polymer therapeutics.[2]

There have been numerous reports in drug and gene delivery fields where many types of block copolymer were synthesized, consisting of poly(ethylene glycol) (PEG) or poly(amino acid) (PAA) derivatives as a polymer segment for particular applications owing to their high biocompatibility. PEG is a nontoxic, hydrophilic polymer with low interfacial free energy in water and high-chain mobility inducing effective steric stabilization effects.[3] For example, it is well-documented that exogeneous proteins appreciably reduce their antigenicity through PEGlyation.[4] In addition, the particular advantages of using PAA as biomaterial with therapeutic interest are the tailored molecular design through the precisely controlled-polymerization using the *N*-carboxy anhydride (NCA) ring open-

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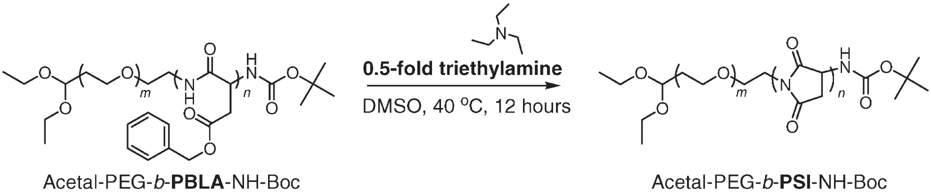
ing method, the availability of a variety of functional groups, such as the carboxyl group and amino group, low toxicity, biodegradability and the formation of characteristic protein folding motif due to inter- and intramolecular association of PAA strands.[5] Therefore, it is highly expected that block copolymers, which are composed of both PEG and PAA segments, show an appreciable biocompatibility and functionality essential for polymer therapeutics.

We have recently established the novel synthetic route of heterotelechelic amphiphilic PEG-PAA block copolymers useful for designing polymeric micelles and vesicles as polymer therapeutics application.[6] Ethylene oxide was anionically polymerized using potassium 3,3-diethoxypropanolate as the initiator,[7] followed by the conversion of hydroxyl terminal to amino group to obtain the heterotelechelic α-acetal-ω-amino PEG (Acetal-PEG-NH_2). A variety of L-α-amino-acid *N*-carboxyanhydrides were block copolymerized by living anionic ring opening polymerization initiated with Acetal-PEG-NH_2 as a macroinitiator to obtain heterotelechelic Acetal-PEG-PAA-NH_2 block copolymer, characterized as having a narrow molecular weight distribution (MWD). The acetal group at the PEG chain end can be converted into an aldehyde group by acidic hydrolysis[8] and may be useful to prepare the site-specifically targetable drug carrier by installing biospecific ligands, including sugars and peptides, on the periphery.[9] The amino group at the PAA chain end is useful as a connecting part of the functionality such as detective probes including fluorescent dyes.

PEG-PAA block copolymers can be further functionalized via the chemical modification by the side chain reaction of PAA to increase their feasibility by binding drugs[10] or probes into the side chain. Although there have been several studies on side-chain modification using poly (lysine), poly(glutamate) or poly(aspartate) as a platform polymer, the side chain reaction of these PAAs does not proceed quantitatively.[11] Alternatively, poly(succinimide) (PSI) has been investigated as an active precursor to preparing the poly (aspartic acid) by hydrolysis[12] and the isomeric library of poly(aspartamide) by the quantitative introduction of functional molecules.[13] Thus, heterotelechelic PEG-PSI block copolymer with a narrow MWD can be an active precursor polymer with a potential for further functionalization. Although a method to prepare a comb-type graft copolymer of PSI with PEG is reported,[14] there is currently no described method for an effective and convenient preparation of PSI homopolymer or PEG-PSI block copolymer with a narrow MWD. This is because PSI is afforded only by the polycondensation of aspartic acid with phosphoric acid,[15] and, as a general rule, polymers prepared by polycondensation are characterized as having a broad MWD. Moreover, the synthesis of PSI has some more drawbacks, including high reaction temperature, branching of the primary structure as a side reaction and coloring of the obtained product.[15] These disadvantages limit the resultant polymer under control.

This paper reports a novel synthetic route to prepare the amphiphlic PEG-PSI block copolymer possessing both acetal and *tert*-butoxycarbonyl-amino (Boc-NH) terminals with a narrow and unimodal MWD that may be useful in the field of drug delivery. Recently, we have established that the flanking benzyl ester groups of poly(β-benzyl L-aspartate) (PBLA) undergo a quantitative aminolysis reaction with various primary amine compounds, thus offering a variety of poly(aspartamide)s.[16] And the detailed mechanism of this unique reaction was clarified that the aminolysis reaction of PBLA proceeds involving succinimide ring formation by the catalysis of the amine compound.[17] Therefore, it is expected that PSI can be prepared from the reaction of PBLA with tertiary amine compounds, since tertiary amines can act as a catalyst leading to the formation of the succinimide ring, yet cannot undergo an aminolysis reaction



Scheme 1.

Synthesis of heterotelechelic Acetal-PEG-*b*-PSI-NH-Boc.

because of the lack of a primary amino group (Scheme 1). For that reasons above, both heterotelechelic PEG macroinitiator method and PBLA-aminolysis method were utilized in order to prepare the heterotelechelic PEG-PSI block copolymer from PEG-PBLA block copolymer.

Experimental Part

Materials

β-Benzyl L-aspartate *N*-carboxy-anhydride (BLA-NCA) was obtained from Nippon Oil and Fats (Tokyo, Japan). Dimethylformamide (DMF), methylenechloride (DCM), dimethylsulfoxide (DMSO) and triethylamine (TEA) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were purified by distillation according to the conventional procedure.[18] Deuterated dimethylsulfoxide (DMSO-d_6) was purchased from Aldrich Chemical Co. Ltd (Milwaukee, WI, USA) and was used as received. The other chemicals were used as received.

Methods

The ^{1}H spectra were recorded on a JEOL EX 300 spectrometer (JEOL, Tokyo, Japan) at 300 MHz. Chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane. Weight-average molecular weight (MW) and MWD were estimated using a gel-permeation chromatography (GPC) (TOSOH HLC-8220) system equipped with two TSK gel columns (TSK-gel Super AW4000 and Super AW3000) and an internal refractive index (RI) detector. The columns were eluted with *N*-methyl-pyrrolidone (NMP) containing lithium bromide (LiBr) (50 mM) (0.3 ml min-1) at 40 °C. MW was calibrated with PEG standards (Polymer Laboratories, Ltd., UK). The infrared absorption (IR) spectra were obtained with an IR-550 JASCO spectrophotometer.

Synthesis of *α*-acetal-*ω*-Boc-amino Poly(ethylene glycol)-*block*-poly(*β*-benzyl L-aspartate) (Acetal-PEG-*b*-PBLA-NH-Boc)

Acetal-PEG-*b*-PBLA-NH_2 was prepared by living anionic ring-opening polymerization of BLA-NCA initiated by Acetal-PEG-NH_2 (MW: 6000, M_w/M_n: 1.02), which was prepared as previously reported.[9] Briefly, BLA-NCA (996.88 mg, 4 mmol) was dissolved in DMF (2.4 mL), followed by the addition of DCM (12.6 mL). The solution of Acetal-PEG-NH_2 (600 mg, 0.1 mmol) in DCM (9.0 mL) was added to the solution of BLA-NCA and stirred for 48 hours at 40 °C. The solution was precipitated in ether (3 L) 3 times and Acetal-PEG-*b*-PBLA-NH_2 was collected as a white powder (1.32 g) with a yield of 92.9%. The DP of PBLA segment was calculated to be 40 based on ^{1}H NMR spectroscopy (Figure 2).

(Acetal-PEG-*b*-PBLA-NH_2 (280 mg, 20 μmol) was reacted with 10-fold di-*tert*-butyldicarbonate (43.65 mg, 0.2 mmol) in DMSO (20 mL) at 40 °C for 6 hours. The product was purified by precipitation in ether (500 mL) four times and dried *in vacuo* to obtain Acetal-PEG-*b*-PBLA-NH-Boc as a white solid (210 mg) with a yield of 75%. Acetal-PEG-*b*-PBLA-NH-Boc was confirmed to have a unimodal MWD (M_w/M_n: 1.07) by GPC measurement (Figure 3).

Synthesis of α-acetal-ω-Boc-amino Poly(ethylene glycol)-*block*-poly (succinimide) (Acetal-PEG-*b*-PSI-NH-Boc)

Lyophilized Acetal-PEG-*b*-PBLA-NH-Boc (200 mg, 0.57 mmol of benzyl ester group in PBLA segment) was dissolved in DMSO (20 mL), followed by the reaction with 0.5-fold TEA (0.5 equiv to the residual benzyl ester group in PBLA segment, 29.3 mg, 0.29 mmol) at 40 °C for 12 hours under argon atmosphere. The product was purified by precipitation in ether (500 mL) three times and the polymer was collected as a white powder (121 mg) with a yield of 84%. The polymer thus obtained was characterized by ^{1}H NMR and IR spectroscopies and GPC measurement.

Reaction Velocity Measurement

The rate of succinmide formation was determined by comparison of the intensity of α-CH of PBLA with that of α-CH of PSI and also by comparison of the intensity of CH_2 of the leaving benzyl alcohol with that of benzyl ester based on ^{1}H NMR spectroscopy using DMSO-d_6 as solvent. Acetal-PEG-*b*-PBLA-NH-Boc (7.5 mg, 21.4 μmol of benzyl ester group) was dissolved in DMSO-d_6 (0.75 mL), followed by the reaction with 0.5-fold TEA (0.5 equiv to the residual benzyl ester group in PBLA, 1.08 mg, 10.7 μmol) at 40 °C. ^{1}H NMR analysis was carried out during the reaction. Each integration value ratio was determined by setting the value of C_6H_5- of benzyl group as 5 in all the ^{1}H NMR spectra.

Results and Discussion

It was observed in ^{1}H NMR spectra that in accordance with the gradual decrease in the peak intensity of α-CH of PBLA at 4.6 ppm, an alternative increase in the intensity at 5.3 ppm (data not shown). According to the reported chemical shift values of PSI,[19] it is reasonable to conclude that the peak at 5.3 ppm is assigned to α-CH of PSI. The time traces of the peak changes were summarized in Figure 1. The integration value of α-CH of PSI became ca. 1 at 12 hours. This is consistent with the complete disappearance of the peak corresponding to α-CH of PBLA. Thus, it was confirmed that the succinimide formation was completed within 12 hours. The time-trace of the ^{1}H NMR spectra revealed that the formation of succinimide promptly progressed in DMSO and was completed for 12 hours.

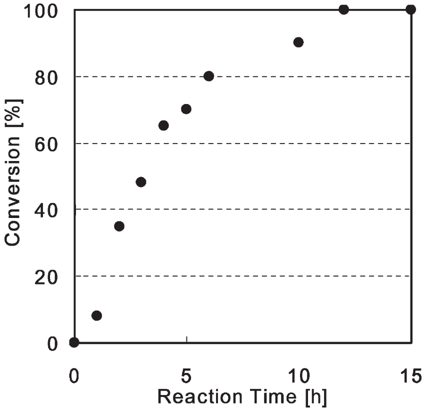


Figure 1.
Time profile of the conversion rate determined by the intensities of α-CHs of BLA and SI residues based on ^{1}H NMR spectroscopy in the condition where Acetal-PEG-*b*-PBLA-NH-Boc reacted with 0.5-fold TEA in DMSO-d_6 at 40 °C.

From ^{1}H NMR measurement, by comparing the peak intensity ratios of CH_3 of acetal group (**a**) with α-CH (**l**) of PSI, the DP of PSI was calculated to be 40, consistent with that of PBLA segment of Acetal-PEG-*b*-PBLA-NH-Boc (Figure 2). The peak intensity ratios of CH_3 of Boc (**k**), CH_2CH_2 of PEG (**d**) and α-CH of PSI (**l**) were mostly identical to those of CH_3 of Boc (**k**), CH_2CH_2 of PEG (**d**) and α-CH of PBLA (**g**), which were calculated by setting the value of CH_3 of acetal group (**a**) as 6 in all the ^{1}H NMR spectra. Figure 3 shows the GPC traces of the resulting polymer and it was confirmed that Acetal-PEG-*b*-PSI-NH-Boc has a unimodal and narrow MWD (M_w/M_n: 1.07), which was in a good agreement with that of Acetal-PEG-*b*-PBLA-NH-Boc. These data demonstrate that the ring formation of BLA residue proceeded successfully without the undesirable side

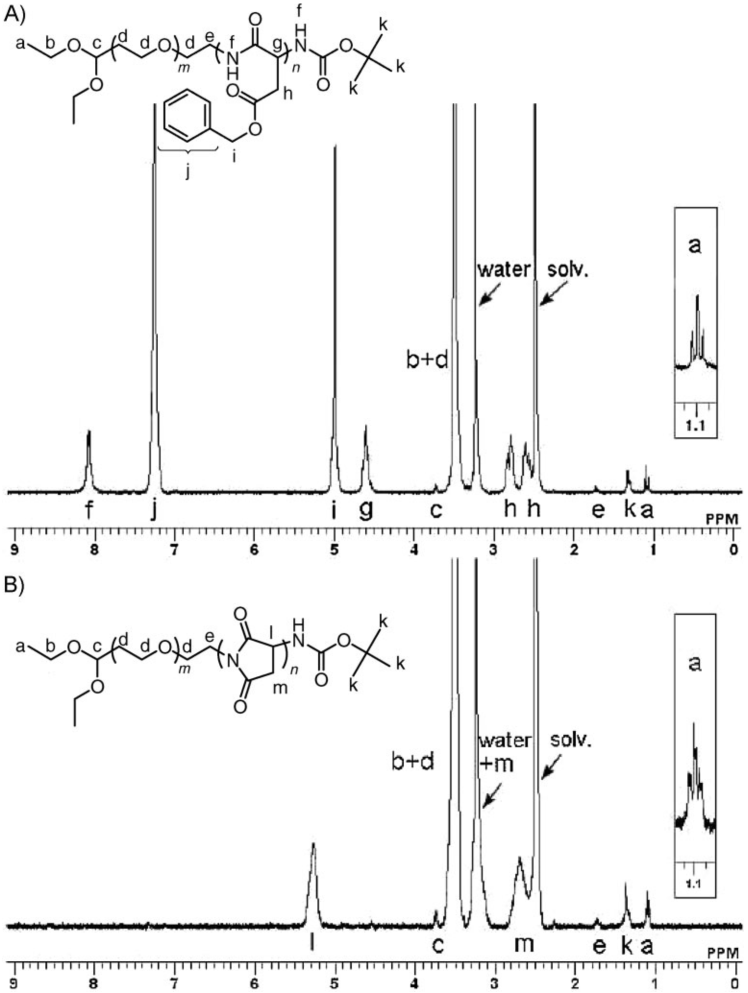


Figure 2.
^{1}H NMR spectra of (A) Acetal-PEG-*b*-PBLA-NH-Boc and (B) Acetal-PEG-*b*-PSI-NH-Boc (solvent DMSO-d_6; concentration, 10 mg mL^{-1}; temperature, 60 °C).

reaction of the cleavage of main chain in the appropriate condition.

Next, an IR measurement was performed to directly detect the succinimide structure. In the IR spectrum of the product, the amide I and ester peaks almost disappeared and the imide peak of the succinimidyl ring appeared clearly at 1716 cm^{-1}, as shown in Figure 4. The IR spectrum of the product was in accordance with the spectrum of PSI which has been reported.[20] Therefore, it is reasonable to conclude that TEA catalytically transduces the BLA residue to succinimide.

Based on the results above, the mechanism is explained as follows; the ring closing reaction of BLA residue starts with the activation of the nitrogen atom in the main chain by TEA as a weak base coordinating the proton of the amide group, and then the nucleophilic attack by the activated nitrogen on the carbon atom of the carbonyl group in the side chain occurs to form the succinimidyl ring. The eliminated proton which recombines with the benzyloxyl group is released as benzyl alcohol, accompanying the regeneration of TEA (Scheme 2). Therefore, TEA works as a

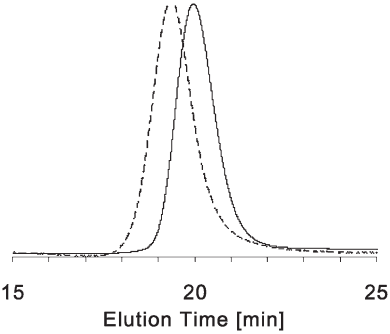
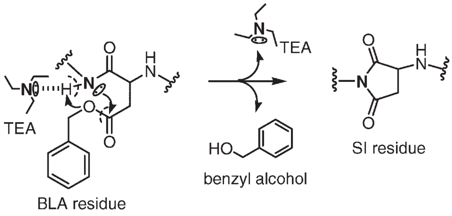


Figure 3.
GPC diagrams of (—) Acetal-PEG-*b*-PSI-NH-Boc and (---) Acetal-PEG-*b*-PBLA-NH-Boc (PEG standard; eluent, NMP containing 50 mM LiBr; temperature, 40 °C; flow rate, 0.3 mL min^{-1}, RI detection).



Scheme 2.
Mechanism of the ring closing reaction of BLA residue by the nucleophilic attack of TEA to form SI residue.

catalyst during the ring closing reaction. The essential point in this reaction is that the nitrogen atom of amide group in the main chain attacks to the carbon atom of the γ-carbonyl of PBLA in the side chain which exits four-atom apart from the nitrogen to form five-membered ring as reported in many papers[12,17,20] (Scheme 2). Thus, this method can be applied for preparing not only the heterotelechelic PSI homopolymer, a variety of PSI block copolymers but also other types of PSI copolymer with PAA segment such as random, graft, star or dendritic copolymers from those types of PBLA-PAA copolymers.

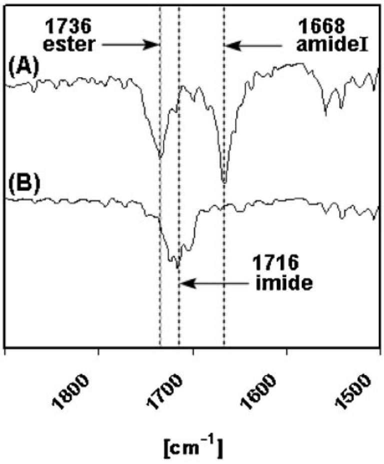


Figure 4.
IR spectra with assignments at a range of 1800–1500 cm^{-1} of (A) Acetal-PEG-*b*-PBLA-NH-Boc and (B) Acetal-PEG-*b*-PSI-NH-Boc.

Conclusion

It was revealed that heterotelechelic Acetal-PEG-*b*-PSI-NH-Boc with a narrow MWD was successfully prepared by the ring closing reaction of PBLA segment with TEA as a catalyst in the mild condition. In conclusion, this method of the succinimide transduction is a simple and efficient way to prepare the various types of PSI copolymers. As for the extension of this study, the preparation of the polymeric micelle from Acetal-PEG-*b*-PSI-NH-Boc and its derivatives is underway in our laboratory, and the results will be reported elsewhere in the near future.

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