Molar Mass and Structural Characteristics of Poly[(lactide-*co*-(aspartic acid)] Block Copolymers

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Summary: We report on various synthetic procedures for the preparation of biodegradable and biocompatible poly(lactide-*co*-aspartic acid) block copolymers based on natural monomeric units – lactic acid and aspartic acid. Multiblock poly(lactide-*co*-aspartic acid) copolymers of different comonomer composition were synthesized by heating a mixture of L-aspartic acid and L,L-lactide in melt without the addition of any catalyst or solvent and with further alkaline hydrolysis of the cyclic succinimide rings to aspartic acid units. Diblock poly(lactide-*co*-aspartic acid) copolymers with different block lengths were prepared by copolymerization of amino terminated poly(β-benzyl-L-aspartate) homopolymer and L,L-lactide with subsequent deprotection of the benzyl protected carboxyl group by hydrogenolysis. The differences in the structure, composition, molar mass characteristics, and water-solubility of the synthesized multiblock and diblock poly(lactide-*co*-aspartic acid) copolymers are discussed.

Keywords: amphiphiles; biodegradable; block copolymers; drug delivery systems; molar mass distribution; structure

Introduction

Poly(lactic acid) or poly(lactide) is a biodegradable, biocompatible and bioabsorbable polyester, which has received much attention in medical, pharmaceutical and packaging applications.[1–10] However, the application scope of poly(lactide) is limited since it is very hydrophobic polymer with no functional groups. In addition, the hydrolytic degradation rate of poly(lactide) for applications for drug delivery purposes is too slow due to its high crystallinity, which results in poorer soft tissue compatibility. To overcome the drawbacks of the poly(lactide) homopolymer, many kinds of hydrophilic comonomer units have been incorporated into the poly(lactide) chain. For this purpose many random, block or graft copolymers have been synthesized from hydrophobic lactide and hydrophilic comonomers such as ethylene oxide, caprolactone, saccharides, amino acids, cellulose, chitosan, etc.[10–24]

In our work we focused on biodegradable and biocompatible poly(lactide-*co*-aspartic acid) (PLAA) copolymers, which are promising amphiphilic polymers for drug delivery applications. The copolymers of lactide and aspartic acid were chosen since they combine the advantages of both poly(lactide) and poly(aspartic acid) derivatives, and hydrolytically degrade to nontoxic bioabsorbable products. The aspartic acid units with carboxyl functional groups serve as a chelating agent for other substances, and enable solubility of the copolymers in water.

Multiblock copolymers of different chemical composition and with a partially branched structure have been prepared from L,L-lactide and L-aspartic acid in melt without using any catalysts or solvents.[12] Diblock poly(lactide-*co*-aspartic acid) copolymers have been synthesized by copolymerization of the β-benzyl-L-aspartate-*N*-

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carboxyanhydride monomer and the amino terminated poly(lactide), which acts as the macroinitiator.[10,16] Adjusting the initial monomer/macroinitiator ratio can control the degree of polymerization of the polypeptide block. The molar mass distribution of the amino terminated poly(lactide) block was rather broad (polydispersity index, PDI $\leq$ 1.5), whereas the authors did not report the PDI values of the poly(lactide-*co*-aspartic acid).

In this contribution we present the preparation of amphiphilic multiblock and diblock poly(lactide-*co*-aspartic acid) copolymers of different comonomer composition as well as the differences in their structure and molar mass characteristics. Multiblock PLAA copolymers were prepared by heating a mixture of L-aspartic acid and L,L-lactide in melt without additional catalysts, whereas the diblock PLAA copolymers were synthesized by controlled copolymerization of the amino terminated poly(β-benzyl-L-aspartate) homopolymer and L,L-lactide in THF/dioxane solution. The differences in structure, composition, molar mass characteristics, and water-solubility of poly(lactide-*co*-aspartic acid) copolymers prepared according to the described synthetic procedures are discussed.

Experimental Part

Materials

Amino terminated poly(β-benzyl-L-aspartate) (PBA-NH_2) was prepared from *N*-carboxy-β-benzyl-L-aspartate anhydride (Asp(OBz)-NCA) according to references 31 and 32. L-Aspartic acid, (Asp, 98%, Aldrich), L,L-lactide, which is a cyclic dimer of L-lactic acid (98%, Aldrich), tetrahydrofuran (THF, water content $<$ 0.05%, Merck), hexane ($\leq$99%, Merck), dioxane ($\leq$99.5%, Merck), *N*,*N*-dimethylacetamide (DMAc, water content $\leq$ 0.03%, Fluka), lithium bromide (LiBr $\leq$ 99.9%, Sigma-Aldrich), tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, 95%, Aldrich), NaOH ($\leq$99%, Merck), and Pd/C (palladium, 10 wt % on activated carbon).

Synthesis

Synthesis of Poly(lactide-co-aspartic acid) Multiblock Copolymers (multiblock PLAA)

Poly(lactide-*co*-succinimide) (PLS) copolymers were synthesized from L-Aspartic acid and L,L-lactide in melt according to the procedure described in reference[12]. The obtained PLS copolymers contain aspartic acid units mainly in the cyclic succinimide form. In the next step the cyclic succinimide rings were converted into more hydrophilic aspartic acid units bearing carboxylate groups by hydrolysis in aqueous NaOH.

Synthesis of Poly(lactide-co-asparic acid) Diblock Copolymers (diblock PLAA)

The *N*-carboxy-β-benzyl-L-aspartate anhydride (Asp(OBz)-NCA), which was chosen as the monomer for the preparation of the polypeptide block, was synthesized as described in the literature.[25] Amino terminated poly(β-benzyl-L-aspartate) (PBA-NH_2) homopolymers were prepared by polymerization of Asp(OBz)-NCA monomer in dry N,N-dimethylformamide (DMF) at room temperature under an argon atmosphere using the primary amine as the initiator according to the procedure described in the reference[26]. By changing the Asp(OBz)-NCA/amine (monomer/initiator) molar ratio in the feed we synthesized PBA-NH_2 homopolymers of different molar masses and low polydispersity indices ($\overline{M}_n$: 2800 and 5700 g mol^{-1}, PDI $\leq$ 1.1).

Poly(L,L-lactide-*co*-β-benzyl-L-aspartate) (PLBA) diblock copolymers were synthesized in a dry box ($O_2 \leq 0.1$ ppm; $H_2O \leq 0.1$ ppm) from PBA-NH_2 and L,L-lactide in a THF/dioxane solution. PBA-NH_2 (0.190 g, 0.068 mmol–0.033 mmol for PBA-NH_2 with molar mass 2800 and 5700 g mol^{-1}, respectively) was put in a three necked glass flask equipped with a mechanical stirrer and contact thermometer, and dissolved in 10 mL of dry THF/1,4-dioxane mixture (1:1 = v/v) during stirring at room temperature. After the PBA-NH_2 homopolymer had dissolved, a solution with a

corresponding amount of L,L-lactide (1.083–0.271 g; 7.528–1.914 mmol) in THF and 0.05 mL of a 0.75 M solution of $Sn(Oct)_2$ were added. The reaction mixture was heated to the predetermined temperature (55, 60, 65, or 75 °C) and stirred for 6 h. The product was precipitated in cold hexane and washed several times with methanol to remove $Sn(Oct)_2$ and unreacted L,L-lactide monomer. The product was then filtered and dried at room temperature in a vacuum oven. In the final step the benzyl groups of PLBA copolymers were removed by hydrogenolysis with palladium (Pd/C). The ^{1}H NMR spectra of PLA diblock copolymers do not show benzyl group signals.

Characterization

NMR Spectroscopy

The composition and structure of copolymers were determined by ^{1}H NMR spectrometry using a Unity Inova 300 Varian NMR spectrometer operating at 300 MHz. The sample concentrations were 1% (w/w) in the solvent DMSO-d_6. All spectra were obtained at 25 °C, and tetramethylsilane (TMS) was used as the internal standard. The conditions for ^{1}H NMR were: a 90° pulse angle, a 5 s delay between pulses, an acquisition time of 5 s, and up to 100 repetitions. For the determination of composition of PLS copolymers ^{1}H NMR spectra were recorded in DMSO-d_6 with added CF_3COOH, which shifts the signal for water to a lower magnetic field and does not overlap with that of the succinimide methylene groups.

SEC-MALS Measurements

The SEC-MALS measurements of samples were performed at 25 °C using a Hewlett Packard pump series 1100 coupled to a Dawn HELEOS laser photometer with a GaAs laser ($\lambda_0 = 656$ nm) and to an Optilab rEX interferometric refractometer operating at the same wavelength as the Dawn HELEOS photometer (both instruments are from Wyatt Technology Corp., USA). Separations were carried out using a 3 μm MesoPore column (300 mm length and 7.5 mm i.d., Polymer Laboratories) with a precolumn in a solution of 0.05 M LiBr in DMAc. The nominal flow rate of eluent was 0.5 mL min^{-1}. The mass of the samples injected onto the column was typically 5×10^{-4} g. Data acquisition and evaluation were carried out using Astra 5.3 software (Wyatt Technology Corp.).

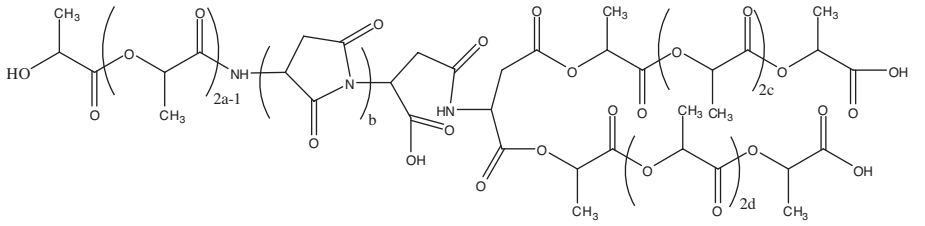
The molar mass averages and molar mass distribution of PLS copolymers were determined using the same chromatographic conditions as in the case of PLBA copolymers, only that they were calculated according to polystyrene (PS) calibration.

Results and Discussion

Multiblock Poly(lactide-*co*-aspartic acid) Copolymers (PLAA)

The determination of poly(lactide-*co*-succinimide) (PLS) structure by NMR spectrometry indicates that the aspartic acid units are mainly in the cyclic succinimide form. The copolymers contain a certain amount of carboxylic acid groups in the aspartic acid units originating from ring opened succinimide sequences. Some carboxyl groups are also present in the lactide sequences as a result of the partly branched structure (Scheme 1).

The composition of PLS copolymers was determined by ^{1}H NMR spectroscopy by comparing the intensities of the proton signals for the methyl group of the lactide units (1.47 ppm) and for the methylene group of the succinimide units (2.6–3.6 ppm). The composition can be altered to a certain degree by changing the initial ratio of both monomers in the feed. However, the highest amount of incorporated succinimide units in PLS is restricted by the solubility of Asp in the L,L-lactide melt. For this reason, the succinimide sequences in PLS are much shorter than the lactide sequences. Relative molar mass averages of the synthesized PLS copolymers were in the range of 10^3–10^4, whereas the molar mass distribution of blocks was broad as reflected by the high values of the PDIs (around 2).



Scheme 1.
Schematic structure of multiblock PLS copolymers.

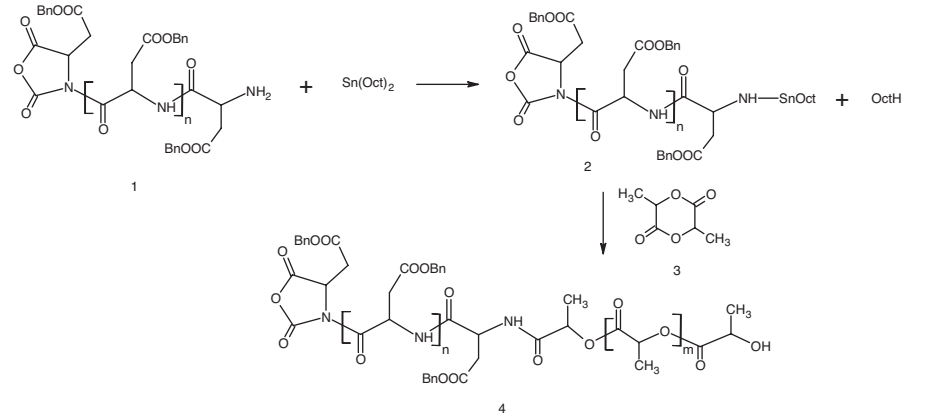
After hydrolysis of PLS copolymers in aqueous NaOH the aspartic acid blocks in PLAA consist of both α- and β- amide linkages. Since the structure of multiblock PLAA is partially branched the carboxylate groups are placed not only in aspartate units but also at the end of lactide branches. For this reason and due to the fact that the limit comonomer composition obtained by melt copolymerization of L,L-lactide and L-aspartic acid without the catalyst was around 2.5:1 in favor of lactide units, the functionality of multiblock PLAAs was not high enough to enable their complete solubility in water.

Diblock Poly(lactide-*co*-aspartic acid) Copolymers (PLAA)

Amino terminated PBA-NH_2 homopolymers of different molar masses were synthesized by changing the Asp-NCA/amine (monomer/initiator) molar ratio in the feed. Since a benzyl group protects the carboxyl group of the Asp-NCA monomer, the possibility of a side reaction leading to branching was to a large extent prevented. The molar mass averages of PBA-NH_2 determined by SEC-MALS were in the range of 10^3 gmol^{-1} and their molar mass distribution was narrow (PDI ≤ 1.1).

Diblock poly(L,L-lactide-*co*-β-benzyl-L-aspartate) (PLBA) copolymers (4) were prepared by ring opening polymerization of L,L-lactide (3) in THF/dioxane solution using the $SnOct_2$ and the amino terminated PBA-NH_2 homopolymer (1) as the initiator and co-initiator, respectively (Scheme 2). The reaction was carried at various temperatures between 55 and 75 °C.

The rate of copolymerization of the L,L-lactide monomer and the yield of the reaction increase as the temperature increases. However, the degree of carboxyl group deprotection also increases with increasing temperatures as indicated by the



Scheme 2.
Synthetic pathway for preparation of PLBA diblock copolymer.

increasing intensity of the signal for –COOH groups at 13–13.5 ppm in the ^{1}H NMR spectra of PLBA copolymers (Figure 1, magnified region). Since free carboxyl groups can react with PBA-NH_2 amino end groups and/or initiate the growth of the poly(lactide) arms we expected that PLBA synthesized at higher temperatures would exhibit some degree of branching. Further synthesis of PLBA copolymers was therefore performed at a reaction temperature of 60 °C, which enables a reasonable rate of copolymerization while still leading to a insignificant degree of carboxyl group deprotection and thus to a negligible level of branching.

The comonomer composition of PLBA copolymers was determined from their ^{1}H NMR spectra by comparing the intensity of the proton signals of the lactide methyl group (–C<u>H</u>$_3$) at 1.47 ppm to that of the aspartate methine group (–C<u>H</u>–) at 4.6 ppm. At a reaction temperature of 60 °C, the degree of polymerization of the lactide block is higher at higher L,L-lactide/PBA-NH_2:$SnOct_2$ (monomer/macroinitiator) molar ratios in the feed and also increases with longer reaction time. The ring opening polymerization of L,L-lactide does not go to completion since equilibrium between growing species and unreacted L,L-lactide monomer is established, which prevents further growth of the poly(L,L-lactide) block. Namely, at a given temperature the ring strain defines the Gibbs' energy of copolymerization. Thus, the less strained six-membered rings such as L,L-lactide reach equilibrium at rather high monomer concentrations.[7]

The molar mass averages and PDI values of PLBA copolymers ($4.8 \times 10^3 < \overline{M}_n < 1.4 \times 10^4$ g mol^{-1}; PDI ≤ 1.3) were higher than those of the starting PBA-NH_2 homopolymers. However, the PDI values of diblock PLBA (PDI ≤ 1.3) were significantly lower than those of multiblock PLS copolymers (PDI around 2), meaning that diblock PLBA copolymers have well defined lengths of both blocks. In addition, after the deprotection of carboxyl groups the aspartic acid block of PLAA copolymers consists of only β-amide linkages. The composition of diblock copolymers, i.e. the length of the peptide and lactide block, can be easily tuned by changing the feed molar ratio of Asp-NCA/amine (monomer/initiator) and L,L-lactide/PBA-NH_2:$SnOct_2$ (monomer/macroinitiator), respectively, such that after carboxyl group deprotection by hydrogenolysis the diblock PLAA copolymers are water-soluble.

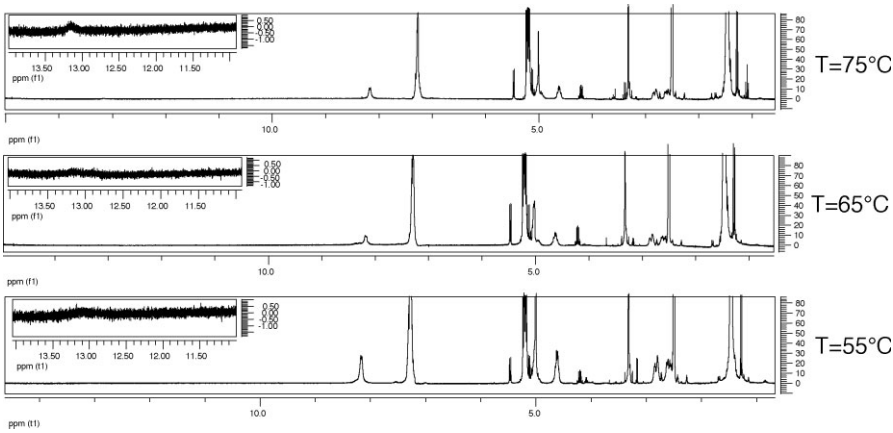


Figure 1.
^{1}H NMR spectra of PLBA diblock copolymers synthesized at different reaction temperatures; magnified regions correspond to the protons of free carboxylic groups formed by hydrolysis of the pendant benzyl ester groups of aspartic acid units.

Conclusion

Multiblok poly(lactide-*co*-succinimide) copolymers synthesized by polycondensation of L-aspartic acid and L,L-lactide in melt have a partially branched structure. Linear diblock poly(L,L-lactide-*co*-β-benzyl-L-aspartate) copolymers were prepared in solution by ring opening polymerization of L,L-lactide on an amino terminated poly(β-benzyl L-aspartate) homopolymer using $SnOct_2$ as the initiator. Both copolymers have comparable molar mass averages, but they differ in molar mass distribution, which is narrower for diblock PLBA copolymers. In addition, the composition can be tuned to a larger extent by solution copolymerization of L,L-lactide and PBA-NH_2 than by polycondensation of L-aspartic acid and L,L-lactide in melt. The aspartic acid block of diblock PLAA copolymers consists only of β-amide linkages, whereas the aspartic acid units in multiblock PLAA obtained by hydrolysis of PLS succinimide rings are linked via α- and β- amide bonds.

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