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Synthesis of 3- and 4- Arm Star-Block Copolypeptides using Multifunctional Amino Initiators and High Vacuum Techniques

Thrasyvoulos Aliferis,[1] *Hermis Iatrou,*[1] *Nikos Hadjichristidis,**[1] *Jamie Messman,*[2] *Jimmy Mays*[2,3]

Summary: Three- and four-arm star-block copolypeptides, $(BL\text{-}b\text{-}G)_3$ and $(G\text{-}b\text{-}BL)_4$, of poly(ε-butyloxycarbonyl-L-lysine) (BL) and poly(γ-benzyl-L-glutamate) (G), were synthesized under high vacuum conditions, using three different multifunctional amino-initiators for the ring opening polymerization of the corresponding α-aminoacid N-carboxyanhydrides. When the initiator contained only primary amine groups {2(aminomethyl)-2-methyl-1,3-propanediamine, AMPDA}, well-defined star-block copolypeptides with monomodal molecular weight distribution were produced. However, if the multifunctional initiator contained both primary and tertiary amine groups {polypropylenimine tetraamine dendrimer, generation 1.0 (DAB); amidoethylethanolamine dendrimer, 1,4-diaminobutane core, generation 0.0 (PAMAM)}, bimodal high polydispersed copolypeptides were formed. The different behaviors are due to the participation of both the primary and tertiary amino groups, each following different polymerization kinetics.

Keywords: α-aminoacid N-carboxyanhydrites; biopolymers; living ring opening polymerization; multifunctional amine initiators; star polymers

Introduction

Recently, an increasing interest in polypeptide-based materials has developed, mainly because of their biocompatibility and the unique ability of polypeptides to organize into α-helix and β-sheet motifs.[1] These motifs are the basis for the richer variety of self-assembled structures in amphiphilic block copolypeptides than those formed from conventional block copolymers. It has also been shown that the physical parameters governing the self-assembly of the branched block copolymers are completely different from those of the corresponding linear analogues.[2] Therefore, the synthesis of star-block copolypeptides, the simplest branched architecture, adds another dimension to the suprastructural hierarchy of biomacromolecules.

Three general synthetic methodologies have been developed for the synthesis of star polymers: a. the use of multifunctional initiators; b. the use of linking agents; and c. the use of difunctional monomers.[3] The advantages of the first approach are the control over the number of arms and the ability to characterize the arms before the linking reaction. The drawbacks are the long time required for the linking reaction, along with the need for fractionation in order to remove the excess living arm added to ensure complete linking. Although the second method does not require fractionation, characterization of the arms, in most cases, is not feasible. In addition, identical polymerization kinetics of each

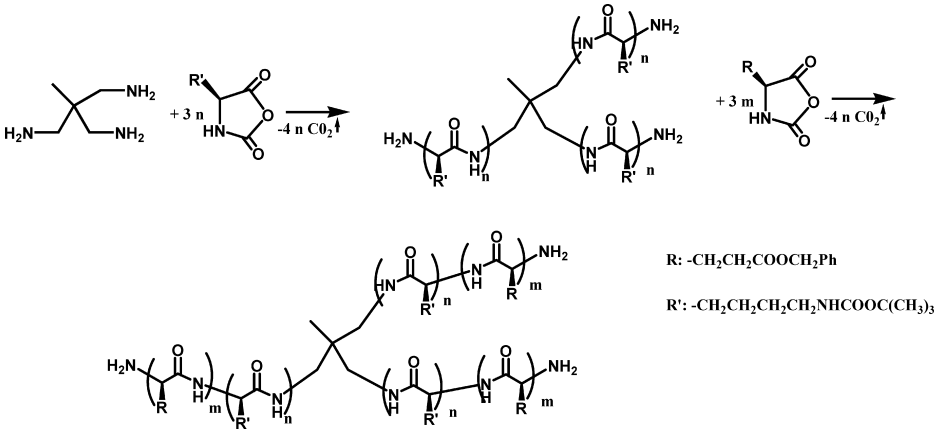
[1] Department of Chemistry, University of Athens, Panepistimiopolis, Zografou, 15771, Athens, Greece
Fax: (+30) 210 7221800
E-mail: hadjichristidis@chem.uoa.gr
[2] Chemical Sciences Division and Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA
[3] Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996, USA

active site should be ensured. In the third method, a living polymer precursor is used as initiator for the polymerization of a small amount of a difunctional monomer. Microgel nodules of tightly cross-linked polymer are formed upon polymerization, which serve as the branch point from which the arms emanate. This method has many drawbacks regarding the inability to control the number and dispersity of arms. Moreover, some of the living arms usually remain unreacted, thus requiring fractionation. In all three cases, only living polymerization can lead to star architectures with well-defined molecular characteristics, i.e. number of arms, molecular weight and low polydispersity indices.

For almost sixty years, attempts to synthesize well-defined living polypeptides have been plagued by unwanted side reactions, rendering the controlled synthesis of well-defined multiblock and branched copolypeptides almost impossible.[4–6] Therefore, until now, only limited studies have been reported for the synthesis of star homopolypeptides.[7–12]

To date, two methods have been presented for the synthesis of high molecular weight well-defined polypeptides, both based on the ring opening polymerization (ROP) of N-carboxyanhydrides (NCAs) and both having the characteristics of ''living'' polymerization. One method involves organonickel initiators, which are less accessible to side reactions due to steric and electronic factors.[13] This approach gave rise to various linear polypeptide-based copolymers, including di-, tri- and pentablocks.[14–16] Our group has developed the second method employing n-hexylamine as the initiator and high vacuum (HVT) techniques, to afford high molecular weight, well-defined living polypeptides in ~100 % yield with low polydispersity.[17] The advantage of this methodology is the preservation of the living sites practically indefinitely. By using trifunctional isocyanates as linking agents, model 3-arm star homo and block copolypeptides, G-*b*-L-*b*-G, G_3, L_3, $(GL)_3$ and $(LG)_3$ were synthesized {G is poly(γ-benzyl-L-glutamate) and L poly(ε-benzylocarbonyl-L-Lysine)}.[12]

In this work, the synthesis and characterization of 3- and 4- arm star block copolypeptides, $(BL\text{-}b\text{-}G)_3$ and $(G\text{-}b\text{-}BL)_4$ {BL is poly(ε-butyloxycarbonyl-L-lysine) and G poly(γ-benzyl-L-glutamate), respectively}, using the multifunctional initiator methodology are reported. The initiators, 2(aminomethyl)-2-methyl-1,3-propanediamine (AMPDA) (99 %, Aldrich) (Scheme 1), polypropylenimine tetraamine dendrimer, generation 1.0 (DAB) (99 %, Aldrich) (Scheme 2), amidoethylethanolamine



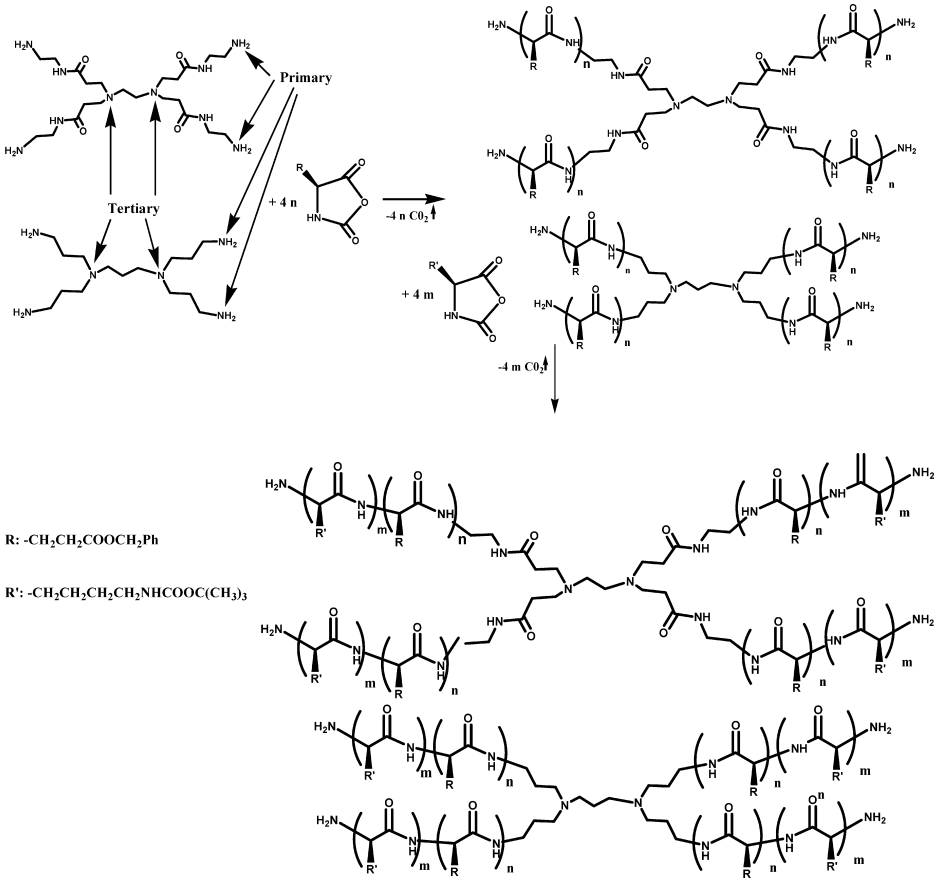
Scheme 1.
General reactions used for the synthesis of 3-arms star copolypeptides using the 2(aminomethyl)-2-methyl-1,3-propanediamine initiator.

dendrimer, 1,4-diaminobutane core, generation 0.0 (PAMAM) (99 %, Aldrich) (Scheme 2), and, were employed in the synthesis and the influence of their chemical natures on the ROP were evaluated.

Experimental

DMF (99.9 + % Acros, special grade for peptide synthesis with less than 50 ppm of active impurities), the polymerization solvent, was further purified by short-path fractional distillation under vacuum in a custom-made apparatus. The middle fraction was always used. The three initiators, DAB, PAMAM and AMPDA, all highly hygroscopic compounds, were left to dry under high vacuum and were then diluted with purified DMF, were subdivided into ampoules and stored under high vacuum at room temperature. NCAs, the monomers, were synthesized from the corresponding α-amino acid and triphosgene in ethyl acetate at 70 °C. Details for the experimental procedures and conditions used for the synthesis of the monomers, along with the high vacuum techniques, are given elsewhere.[17,18]

All polymerizations were performed under high vacuum, in glass reactors equipped with break-seals along with magnets



Scheme 2.
General reactions used for the synthesis of 4-arms star copolypeptides using the amidoethylethanolamine dendrimer, 1,4-diaminobutane core, generation 0.0 (PAMAM) and polypropylenimine tetraamine dendrimer, generation 1.0 (DAB) initiators, respectively.

covered with glass, and constrictions, for the addition of reagents and removal of the intermediate product.

The characterization was performed by size exclusion chromatography (SEC), with a two angles laser light scattering detector (for polydispersity and weight-average molecular weight determinations) and a diode-array UV-VIS detector operating at 267 nm (for analysis of polypeptides with UV-absorbing groups). The SEC was calibrated by low polydispersity PBLG samples, synthesized by high vacuum techniques (HVT) in our laboratory. Membrane osmometry (MO) was also used for the determination of the number average molecular weight of the synthesized materials. Both analyses were performed at 60 °C with a 0.1N LiBr solution in DMF.

Results and Discussion

Synthesis of the 3-arm Star-Block Copolypeptides, $(BL\text{-}b\text{-}G)_3$, with the AMPDA Initiator

The general reactions used for the synthesis of the 3-arm star-block copolypeptides of poly(ε-butyloxycarbonyl-L-lysine) (BL) and poly(γ-benzyl-L-glutamate) (G) with the AMPDA initiator are given in Scheme 1. As an example, the SEC chromatograms following the synthesis of star $(BL_{220}\text{-}b\text{-}G_{150})_3$ are provided in Figure 1. It can be observed that the polydispersity index of $(BL_{220}\text{-}b\text{-}G_{150})_3$ is very low, due to the presence of only primary amines in the initiator, which polymerize the NCAs via the "normal" mechanism.[5] The molecular weights and compositions of the synthesized 3-arm star-block copolypeptides (last two, Table 1) were very close to those expected theoretically, indicating that the copolypeptides synthesized exhibit a high degree of molecular and compositional homogeneity.

Synthesis of the 4-arm Star-Block Copolypeptides, $(G\text{-}b\text{-}BL)_4$, with PAMAM and DAB Initiators

Three different star-block copolypeptides were synthesized (Table 1), using either, the PAMAM or DAB initiators. The first two copolymers were synthesized with PAMAM and the third with DAB. The reaction sequence is shown in Scheme 2.

The SEC chromatogram of the final products of the 4-arms star copolypeptide $(G_{344}\text{-}b\text{-}BL_{270})_4$ reveals a bimodal peak (Figure 2). The main, high molecular weight

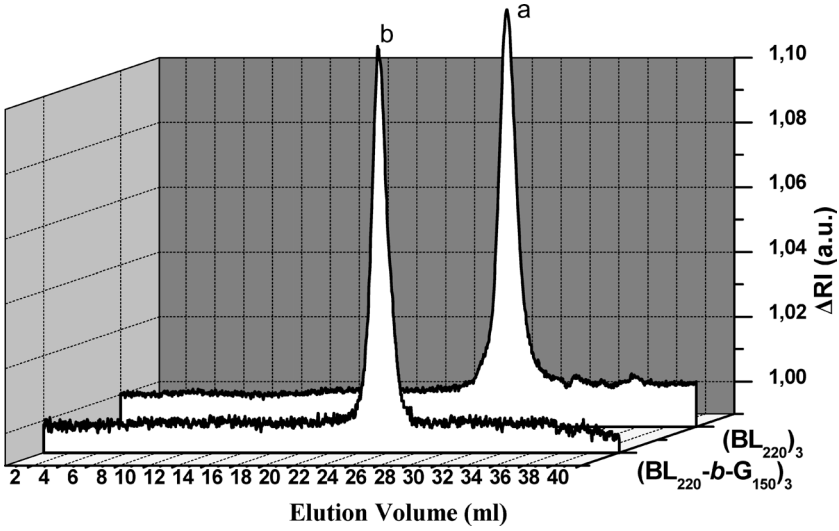


Figure 1.
Monitoring the synthesis of 3-arm star-block $(BL_{220}\text{-}b\text{-}G_{150})_3$ with SEC. a. Precursor $(BL_{220})_3$, b. Star-block copolypetide (crude product).

Table 1.
Molecular characteristics of star block copolypeptides.

Sample	Initiator	M_{nS}[a] $\times 10^3$	M_{nM}[b] $\times 10^3$	I[c]	M_{nTS}[a] $\times 10^3$	M_{nTM}[b] $\times 10^3$	I[c]	%BL[d] (M_n)	% BL[e] (NMR)
$(G_{320}\text{-b-}BL_{100})_4$	PAMAM	71.0	74.4	1.13	93.0	97.8	1.16[f]	24	21
$(G_{330}\text{-b-}BL_{179})_4$	PAMAM	73.0	76.2	1.14	113.0	118.3	1.18[f]	35	33
$(G_{344}\text{-b-}BL_{270})_4$	DAB	76.0	80.1	1.16	135.0	141.6	1.18[f]	44	42
$(BL_{220}\text{-b-}G_{150})_3$	AMPDA	49.0	50.6	1.06	81.0	81.5	1.07[g]	76	74
$(BL_{270}\text{-b-}G_{200})_3$	AMPDA	60.0	60.6	1.07	104.0	102.4	1.07[g]	57	59

[a] Stoichiometric molecular weights
[b] Membrane osmometry, in DMF/LiBr 0.1 N at 60 °C
[c] SEC-LALLS in DMF/LiBr 0.1 N at 60 °C.
[d] Calculated from stoichiometric molecular weights
[e] ^{1}H NMR Spectroscopy in DMSO-d6
[f] After fractionation
[g] Crude product

peak corresponds to the 4-arm star-block copolypeptide resulting from the primary amines. The low molecular weight peak possibly corresponds to linear polypeptides resulting from the tertiary amines, which polymerize NCAs according to the activated monomer mechanism.[5] The star-block copolypeptides corresponding to the main peak were separated from the side products by multiple fractional precipitations, using DMF/LiCl as the solvent/non-solvent pair.

The final star-blocks synthesized by both the PAMAM and the DAB initiators, exhibited significantly higher polydispersity indices than those obtained by the AMPDA initiator, even after fractionation (Table 1). The experimentally determined molecular weights were slightly larger than those theoretically expected, due to the formation of the side products, which modified the monomer to initiator ratio.

Conclusions

Application of the multifunctional initiator methodology allowed for the synthesis of 3- and 4-arm star-block copolypeptides. When the multifunctional initiator was composed of primary and tertiary groups, (PAMAM

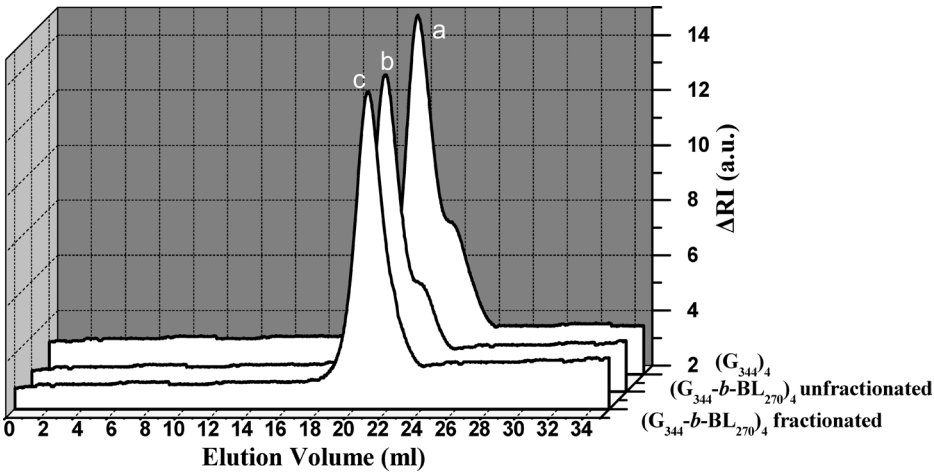


Figure 2.
Monitoring the synthesis of 4-arm star $(G_{344}\text{-b-}BL_{270})_4$ with SEC. a. Precursor 4-arm star homopolymer $(G_{344})_4$, b. Unfractionated star-block copolypeptide c. Fractionated star-block copolypeptides.

and DAB), polypeptides with increased polydispersity indices were produced along with side products. On the contrary, a multifunctional initiator containing only primary amines, (AMPDA) led to well-defined polypeptides with high molecular weight and compositional homogeneity.

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