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A Mechanistic Study of α-(Amino acid)-*N*-carboxyanhydride Polymerization: Comparing Initiation and Termination Events in High-Vacuum and Traditional Polymerization Techniques

Deanna L. Pickel,[†] Nikolaos Politakos,[‡] Apostolos Avgeropoulos,[‡] and Jamie M. Messman*[,†]

[†]*Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, and* [‡]*Department of Materials Science and Engineering, University of Ioannina, University Campus Dourouti, 45110 Ioannina, Greece*

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ABSTRACT: High-vacuum polymerization of α-(amino acid)-*N*-carboxyanhydrides (NCAs) affords polymers with controlled molecular weights and narrow polydispersities; however, a comprehensive study of the end-group composition of the resulting polypeptides has not yet been performed. This reveals crucial information, as the end-groups are indicative of both the polymerization mechanism (i.e., initiation event) and the termination pathways. To this end, poly(*O*-benzyl-L-tyrosine) initiated by 1,6-diaminohexane was synthesized and subsequently characterized by MALDI-TOF MS, NALDI-TOF MS, and ^{13}C NMR spectroscopy to ascertain the end-group structure. Polymers were prepared by both high-vacuum and glovebox techniques in DMF/THF. Preparation of poly(*O*-benzyl-L-tyrosine) by high-vacuum techniques yielded a polymer initiated exclusively by the normal amine mechanism, and termination by reaction with DMF was observed. In contrast, polymers prepared in the glovebox were initiated by the normal amine and activated monomer mechanisms, and several termination products are evident. To our knowledge, this is the first rigorous and comparative analysis of the end-group structure, and it demonstrates the advantage of high-vacuum techniques for polymerization of NCAs for the preparation of well-defined polypeptides with end-group fidelity.

Introduction

Nature creates an incredibly complex and diverse range of structures that provide life-giving function through the precisely tailored synthesis of macromolecules composed of 20 amino acid building blocks. The biological activity of proteins arises from nature's ability to precisely arrange the sequence of amino acids, which in turn determines conformation (coils, α-helix, β-sheet) and governs the creation of well-defined hierarchical structures (tertiary and quaternary structures) through self-assembly.[1] Although synthetic chemists can design and synthesize an enormous variety of macromolecules, a major scientific challenge is to understand how to encode specific structure and function through selection and arrangement of constituent building blocks as well as through changes in macromolecular topology and composition. In this regard, the design of useful biomimetic materials having precisely engineered properties is directly enabled by elucidating the fundamental links between synthesis, structure, properties, and interactions of biomacromolecules.

Poly(amino acid)s or polypeptides are a class of biomimetic materials that have interesting properties resulting from well-defined conformational and hierarchical structures.[2] These properties are dictated in part by factors including chemical composition (i.e., choice of amino acid), sequence of amino acids, and molecular weight. Synthetic polypeptides are prepared from the polymerization of α-(amino acid)-*N*-carboxyanhydrides (NCAs).[1−3] The ability to control the polymerization of NCAs to produce well-defined polypeptides is not trivial because NCAs have multiple reactive centers (two electrophilic and two nucleophilic). The initiation mechanism of an NCA is governed by the initiator's relative nucleophilicity and basicity. The initiator must have sufficient nucleophilicity in order to attack the C5 (C═O) of the NCA, resulting in ring-opening polymerization via the normal amine mechanism; however, if the initiator is significantly basic, deprotonation of the NCA occurs, and the resulting nucleophile initiates chain growth via the activated monomer mechanism (AMM). In the case where primary amines are used to initiate the polymerization of NCAs, two initiation mechanisms are possible: normal amine mechanism (NAM) and activated monomer mechanism (AMM), as shown in Scheme 1. In general, primary amines are more nucleophilic than basic and are efficient NCA polymerization initiators via the NAM mechanism. Additionally, polymerization of NCAs is plagued by a variety of side reactions. These reactions dramatically change the end-group functionality of the polymer and can result in termination of the propagating chain. Historically, such behavior did not allow the controlled polymerization of NCAs initiated by amines.[3]

Recent advances in polymerization techniques have led to the controlled polymerization of NCAs as well as offering the possibility of "living" polymerization of NCAs. Swarcz[4] coined the expression living polymers to describe polymerizations that proceed in the absence of termination and chain transfer reactions. To this end, a substantial amount of work has been done by Kricheldorf,[3] Deming,[5−7] Schlaad,[8,9] and Cheng[10,11] to elucidate the mechanism and "livingness" of NCA polymerizations. It should be noted that each of these groups utilize different chemistries to effectively polymerize NCAs. A common approach is to use primary amines to initiate NCA polymerizations to directly generate amine-terminated polypeptides, which has been discussed comprehensively.[3] Schlaad and co-workers[7,9]

*Corresponding author: Ph (+1)865-576-2394; Fax (+1)865-574-1753; e-mail messmanjm@ornl.gov.

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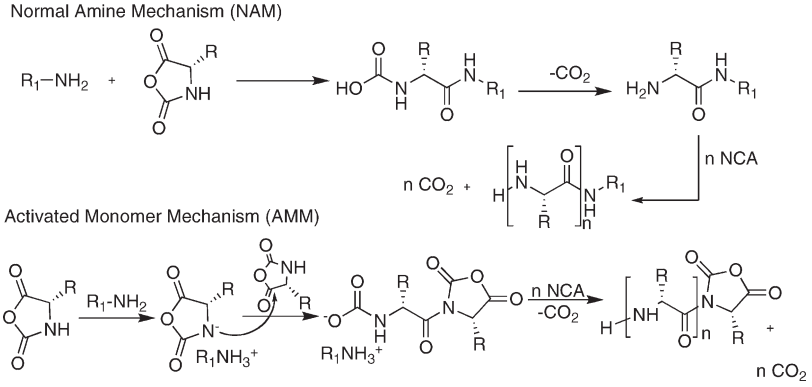
Scheme 1. Normal Amine and Activated Monomer Mechanisms for the Polymerization of NCAs



have utilized primary amine hydrochloride salts as initiators to avoid the formation of NCA anions during the polymerization, presumably inhibiting polymerization via AMM. This particular synthetic approach results in polypeptides containing hydrochloride salts, which require titration with base to obtain the free amine end-group. While they observe low polydispersities for the resulting polymers, no end-group characterization has been done to ascertain whether the AMM mechanism is eliminated by the use of the amine hydrochloride salt initiators. In addition, the molecular weight of the resulting polymers are higher than expected from the monomer to initiator ratio, suggesting some form of termination.[7,9]

A recent publication by the Hadjichristidis[12] group suggests that purification and polymerization of NCAs using high-vacuum techniques is a viable method to produce polypeptides having predictable molecular weights, high degrees of polymerization, and low polydispersities. They hypothesize that the absence of impurities accounts for control over the polymerization. Furthermore, the ability to intermittently remove carbon dioxide (CO_2) generated during the reaction (through a stopcock to the manifold system) effectively mediates the polymerization via NAM and suppresses the influence of the carbamic acid$-CO_2$ equilibrium.[13−15] While low-polydispersity materials of predictable molecular weights are also attained by this approach, the structure of the chain ends has not been investigated. As inferred from Scheme 1, this information is important because chain-end structure of the resulting polypeptides provides direct insight into the operative initiation mechanism(s) (i.e., NAM or AMM) as well as insight into possible termination reactions.

In this regard, Lu and Cheng used *N*-trimethylsilylamines for controlled ring-opening polymerization of NCAs, and end-groups were determined for oligomeric products using ^{13}C NMR spectroscopy and fast atom bombardment mass spectrometry (FAB-MS)[10] as well as electrospray ionization mass spectrometry (ESI-MS).[11] These authors were able to effectively control molecular weight while maintaining narrow molecular weight distribution. One particular difference with this method is that primary amine end-groups are not obtained until postpolymerization chemical modification is used to cleave the protecting trimethylsilyl end-groups.

While end-group characterization of synthetic polypeptides is not trivial, particularly for high molecular weight polymers, Giani and co-workers[16−19] recently utilized nonaqueous capillary electrophoresis (NACE) to characterize the end-group structure of crude polypeptides prepared by conventional Schlenk techniques. NACE allows for the separation and quantification of polymers having different chain ends. For polymerizations of the NCA of N_ε-trifluoroacetyl-L-lysine initiated by hexylamine at 20 °C, the crude product was composed of 78% dead chains (carboxylate and formyl end group), and only 22% of the chains are living (i.e., chains have amine end-groups). A decrease in the polymerization temperature resulted in a product where 99% of the chains are living (i.e., chains have amine end-groups). The authors concluded that the NAM is the only possible initiation pathway and that "dead" polymers result from side reactions of the propagating chain ends with DMF or the NCA. A similar study on polymers prepared by high-vacuum techniques has not been reported.

Therefore, it is desirable to understand the end-group structure of polymers prepared from the primary amine-initiated polymerization of NCAs using high-vacuum techniques. Hadjichristidis et al. have demonstrated characteristics attributed to a living polymerization by monitoring polymerization kinetics of several NCA (co)polymerizations where the degree of polymerization (DP) was large (> 100); they attribute the ability to control the polymerization to the high purity achieved using HVT.[12,21,22] In this report, we have not conducted kinetic measurements of the polymerizations, rather focusing on the resulting polymer structure with considerable attention given to end-group analysis. For this reason, low molecular weight polypeptides are synthesized and studied because low molecular weight materials allow for rigorous end-group characterization by both matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and ^{13}C NMR spectroscopy.[23] While several authors have reported polypeptides with narrow molecular weight distribution (MWD), and included MALDI-TOF MS analysis,[3] to the best of our knowledge, there have been no reports of rigorous end-group analysis (high-resolution ^{13}C NMR and MALDI-TOF MS with isotopic resolution) contrasting the structures of polypeptides from AMM and NAM.

In this article, we show the synthesis and characterization of telechelic poly(*O*-benzyl-L-tyrosine) having predetermined molecular weight, low polydispersity, and retention of primary amine end-groups. The retention of the primary amine end-groups allows for the synthesis of block copolymers by sequential monomer addition as well as other postpolymerization reactions. In addition, it is demonstrated that the polymerization proceeds exclusively via the normal amine mechanism with minimal termination. Polymers prepared by high-vacuum techniques are compared to those prepared in a glovebox environment.

Experimental Section

Materials. Tetrahydrofuran (THF, Fisher, Reagent grade) was treated with Na° followed by distillation onto Na°/K° alloy; purified THF was obtained by vacuum distillation from Na°/K° alloy immediately prior to use. Hexanes (Aldrich, 95% anhydrous) was dried over calcium hydride followed by distillation onto *n*-butyllithium and subsequently vacuum-distilled prior to use. *N,N*-Dimethylformamide (DMF, Fluka, extra dry) was stirred over a ScavengePore benzyl isocyanate resin (Aldrich,

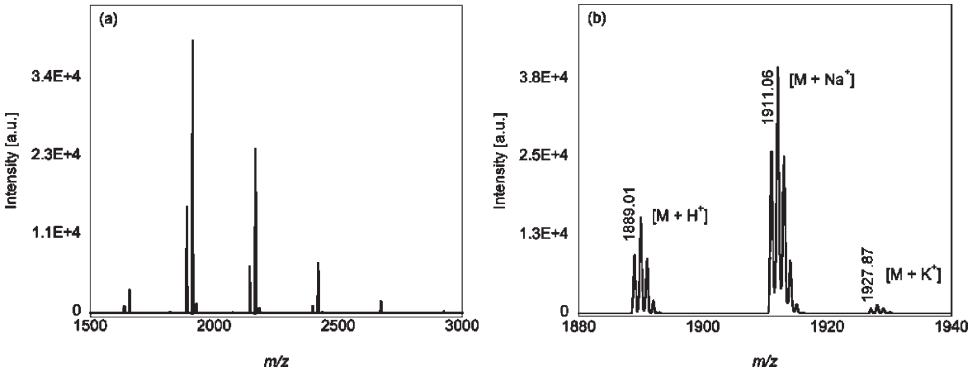


Figure 1. MALDI-TOF MS of HV P(ObLTyr): (a) full spectrum; (b) region corresponding to the 7-mer initiated by DAH.

0.25 g of resin per 200 mL of DMF), filtered, and vacuum-distilled at room temperature prior to use. DMF (Sigma-Aldrich, CHROMASOLV Plus, for HPLC, ≥99.9%) was used as received for glovebox polymerizations. 1,6-Diaminohexane (DAH, Aldrich) was stirred over calcium hydride, followed by stirring over a Na° mirror. It was then distilled into calibrated ampules and flame-sealed from the vacuum line. All other reagents were used as received.

Synthesis. Synthesis of *N*-carboxyanhydride of *O*-benzyl-L-tyrosine: *O*-Benzyl-L-tyrosine *N*-carboxyanhydride (TyrNCA) was prepared by the reaction of *O*-benzyl-L-tyrosine (10.0 g, 33.7 mmol, Acros) with excess phosgene (67.3 mmol, Aldrich, 20% in toluene) in THF under an inert nitrogen environment at 40 °C for 2−3 h. The solvent was removed by vacuum distillation, and the resulting solid was dried overnight on a high-vacuum line. TyrNCA purification was performed in a glovebox under an inert argon atmosphere. In general, the TyrNCA was dissolved in dry THF and subsequently recrystallized from hexanes. In most cases, a 3:1 ratio of hexanes/THF was used. This ratio is adjusted to slowly generate long, needle-like crystals that are nearly colorless. The solvent was decanted from the resulting crystals, and the crystals were vacuum-dried prior to use.

Polymerizations were carried out using both high-vacuum techniques and glovebox techniques. The high-vacuum polymerization of TyrNCA was performed as described by Hadjichristidis.[12] The DAH-initiated polymerization of TyrNCA was effected in all-glass sealed reactors equipped with break-seals at room temperature in THF/DMF. In the reactor, 0.039 g of DAH (0.34 mmol) was dissolved in 40.0 mL of THF and 2.0 mL of DMF. To this, 0.94 g of TyrNCA (3.2 mmol) in THF (5.0 mL) was added by opening a break-seal. Evolved CO_2 was removed intermittently by opening the reactor to the vacuum line via a stopcock. After 24 h, the reactor was opened to the atmosphere, and solvent was removed by evaporation in a fume hood. The resulting polymer is referred to by the abbreviation HV P(ObLTyr). The gravimetric yield of the polymerization is 100%.

Polymerization of TyrNCA was also carried out in the glovebox under argon using purified DMF and DMF as received. In a round-bottom flask, 0.99 g of TyrNCA (3.3 mmol) was dissolved in 45 mL of THF. To this mixture 0.040 g of DAH (0.34 mmol) in 2.1 mL of DMF was added, and the reaction was stirred for 24 h. THF and DMF were removed by evaporation in a fume hood. The resulting polymers are hereafter referred to by the abbreviation GB P(ObLTyr)-1 (purified DMF) and GB P(ObLTyr)-2 (using DMF as received). The gravimetric yield of the polymerizations is 100%.

Characterization. High-temperature size exclusion chromatography (HTSEC) was performed on a Waters GPCV2000 equipped with Viscotek ViscoGEL I-Series columns (1 mixed bed low MW and 2 oligomeric MW in series), a differential refractometer (λ = 880 nm), and a Precision Detectors two-angle light scattering detector (λ = 682 nm) operating at 60 °C. Waters Alliance GPC 2000 software was used to collect data, which was subsequently analyzed using Waters Empower software in which a conventional calibration curve based on low-PDI polystyrene standards (162−5.00 × 10^4 g mol^{-1}) was used to evaluate molecular weight characteristics. DMF containing 0.05 M lithium bromide (LiBr) was used as the eluent at a flow rate of 1.0 mL/min. Matrix-assisted laser desorption-ionization mass spectra (MALDI-TOF MS) were recorded on a Bruker Autoflex II mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with a nitrogen laser operated at 337 nm, a frequency of 5 Hz, and an acceleration voltage of 20 kV. Samples were prepared by the solvent-free method.[24] 2,5-Dihydroxybenzoic acid was used as the matrix and sodium trifluoroacetate as the cationizing agent. Reagents were mixed in a 5:1:1 (matrix:analyte:salt) ratio. Nano-assisted laser desorption−ionization mass spectrometry (NALDI-TOF MS) was performed using a NALDI target (Bruker Daltonics, Billerica, MA). Samples and salts were dissolved in hexafluoroisopropanol (10 mg/mL) and mixed in a 1:1 ratio prior to deposition on the target. Mass spectra were measured in reflectron mode, and the mass scale was externally calibrated with polystyrene standards. ^{13}C NMR spectra were collected on a Varian VNMRS 500 MHz spectrometer with d1 set at 3 s. Samples were dissolved in a 3:1 (vol:vol) mixture of $CDCl_3$ and trifluoroacetic acid.

Results and Discussion

Initiation Reactions. The high-vacuum polymerization of the NCA of *O*-benzyl-L-tyrosine (TyrNCA) was carried out in all-glass sealed reactors as described previously by Hadjichristidis.[12] The polymerization was initiated with 1,6-diaminohexane (DAH), a difunctional initiator, in a mixture of tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) with a target molecular weight of 2800 g/mol. The resulting polymer, HV P(ObLTyr), was characterized by high-temperature size exclusion chromatography (HTSEC) in DMF/LiBr, MALDI-TOF MS, and ^{13}C NMR spectroscopy. By HTSEC, a unimodal, narrow distribution peak was observed, corresponding to a molecular weight of 4200 g/mol and a polydispersity of 1.03, relative to polystyrene standards. The molecular weight determined by HTGPC is not in agreement with the calculated molecular weight, which can be attributed to the difference in hydrodynamic volume of the polystyrene (PS) standards in DMF as compared to the HV P(ObLTyr). In our experience, analysis of polypeptides via HTSEC is oftentimes complicated and can be unreliable insofar that salt concentration (typically LiBr), column type, and polymer concentration affect the separation efficiency and polymer aggregation, which has been observed by others.[26−28]

To assess the chain-end functionality of HV P(ObLTyr), the polymer was analyzed by MALDI-TOF MS, and results

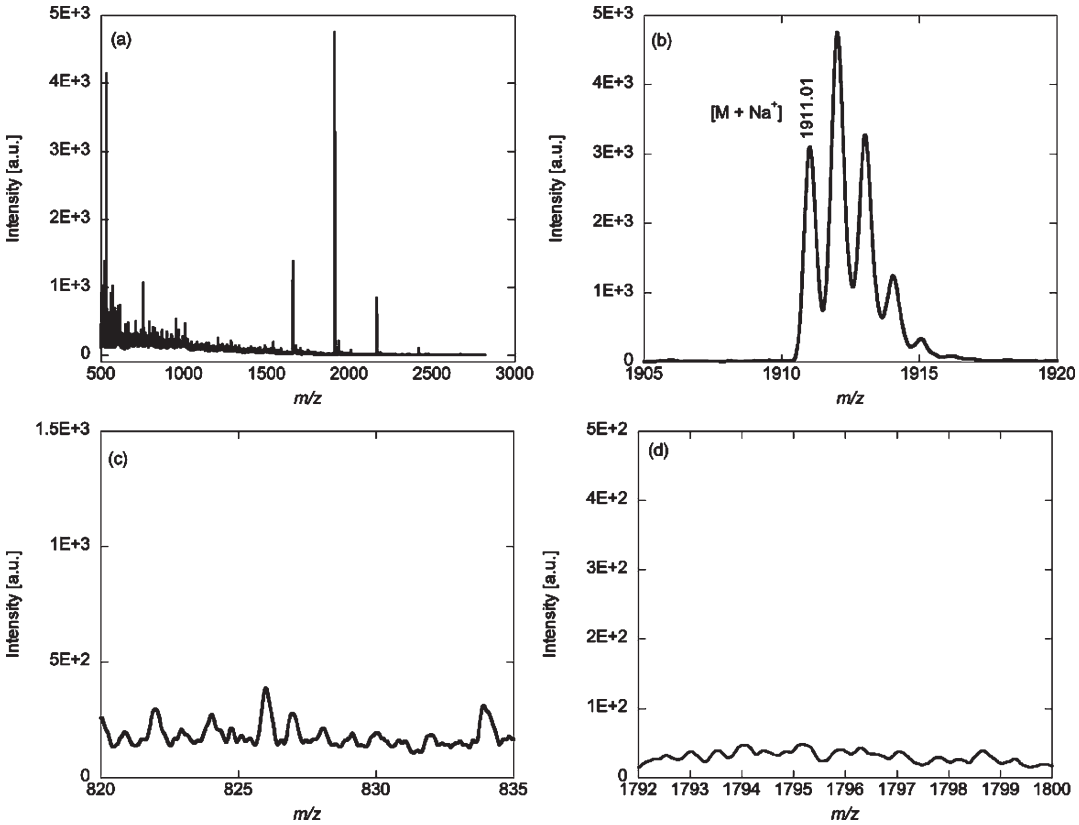


Figure 2. NALDI-TOF MS of HV P(ObLTyr): (a) full spectrum; (b) region of the 7-mer initiated by DAH; (c) region of the 2-mer initiated by AMM; (d) region of the 7-mer initiated by AMM followed by loss of CO_2 by fragmentation.

are shown in Figure 1. The molecular weight determined by MALDI-TOF MS (M_n = 2000 g/mol) is lower than the calculated molecular weight of 2800 g/mol, perhaps due to the MALDI preparation method or due to differences in ionization efficiency of the high molecular weight species.[25] Three distributions were observed in the mass spectrum corresponding to the H^+, Na^+, and K^+ adducts of the desired product, resulting solely from the normal amine mechanism (Figure 1b). For example, the sodiated 7-mer initiated by DAH has a calculated monoisotopic mass of 7×253.1103 ($7 \times C_{16}H_{15}O_2N$) + 114.1157 ($C_6H_{14}N_2$) + $2 \times$ 1.0079 ($2 \times H$) + 22.9898 ($^{23}Na^+$) = 1910.89 Da. No peak is observed for the 7-mer that would be initiated via the activated monomer mechanism, which would have a calculated monoisotopic mass of 7×253.1103 ($7 \times C_{16}H_{15}O_2N$) + 296.0923 ($C_{17}H_{14}O_4N$) + 1.0079 (H) + 22.9898 ($^{23}Na^+$) = 2092.86 Da.

TyrNCA was also polymerized in a glovebox using less stringent techniques in order to show the formation of polymer via the activated monomer mechanism. Polymers were prepared in the glovebox using purified DMF, GB P(ObLTyr)-1, or DMF as received, GB P(ObLTyr)-2. All other reagents were purified similar to the high-vacuum techniques. End-group analysis by MALDI-TOF MS did not indicate the presence of polymer produced via the AMM despite literature precedence.[3,5,29−31] Therefore, a new technique was used for the end-group characterization of the polypeptides, nano-assisted laser desorption/ionization time-of-flight mass spectrometry (NALDI-TOF MS).

NALDI-TOF MS utilizes a target covered with a layer of inorganic nanostructures. These nanostructures absorb laser energy, resulting in laser desorption of the sample without the need for a matrix. The resulting mass spectra are obtained with higher sensitivity and lower chemical background than traditional MALDI-TOF MS.[32] The disadvantage of NALDI-TOF MS is that the upper molecular weight limit is in the range of 2000−3000 g/mol. Therefore, only low molecular weight samples can be analyzed via this method, and true molecular weight distributions cannot be obtained.

Poly(*O*-benzyl-L-tyrosine)s prepared by both high-vacuum techniques and glovebox techniques were analyzed by NALDI-TOF MS. The mass spectrum obtained from HV P(ObLTyr) is shown in Figure 2. The desired product, DAH initiated polymer, is observed as shown in Figure 2b, at m/z = 1911.01. No peak was observed at m/z = 826.31 (Figure 2c), indicating the absence of the AMM (Figure 2c).

Poly(*O*-benzyl-L-tyrosine)s prepared by glovebox methods were also analyzed by NALDI-TOF MS. The mass spectrum for GB P(ObLTyr)-2 where DMF is used as received is shown in Figure 3. The main distribution is identified as the polymer initiated by DAH (Figure 3b) with a calculated monoisotopic mass of 1910.89 Da. A second distribution is also identified as the product resulting from the activated monomer mechanism as shown in Figure 3c. The peak at m/z = 825.14 is attributed to the sodiated 2-mer resulting from AMM with a calculated monoisotopic mass of 2×253.1103 ($2 \times C_{16}H_{15}O_2N$) + 296.0923 ($C_{17}H_{14}O_4N$) + 1.0079 (H) + 22.9898 ($^{23}Na^+$) = 826.31 Da. The corresponding 7-mer is not observed; however, a third distribution that is also attributed to the activated monomer mechanism is shown in Figure 3d. The peak at m/z = 1795.05 corresponds to polymer initiated via AMM followed by loss of CO_2. The calculated monoisotopic mass is 7×253.1103 ($7 \times C_{16}H_{15}O_2N$) + 252.1025 ($1 \times C_{16}H_{14}O_2N$) + 1.0079 (H) + 22.9898 ($^{23}Na^+$) = 1794.76 Da. This distribution most likely results from decarboxylation of the anhydride chain end during ionization.[33] This fragmentation product is observed from the 6-mer up to the 10-mer and was not observed for polymer

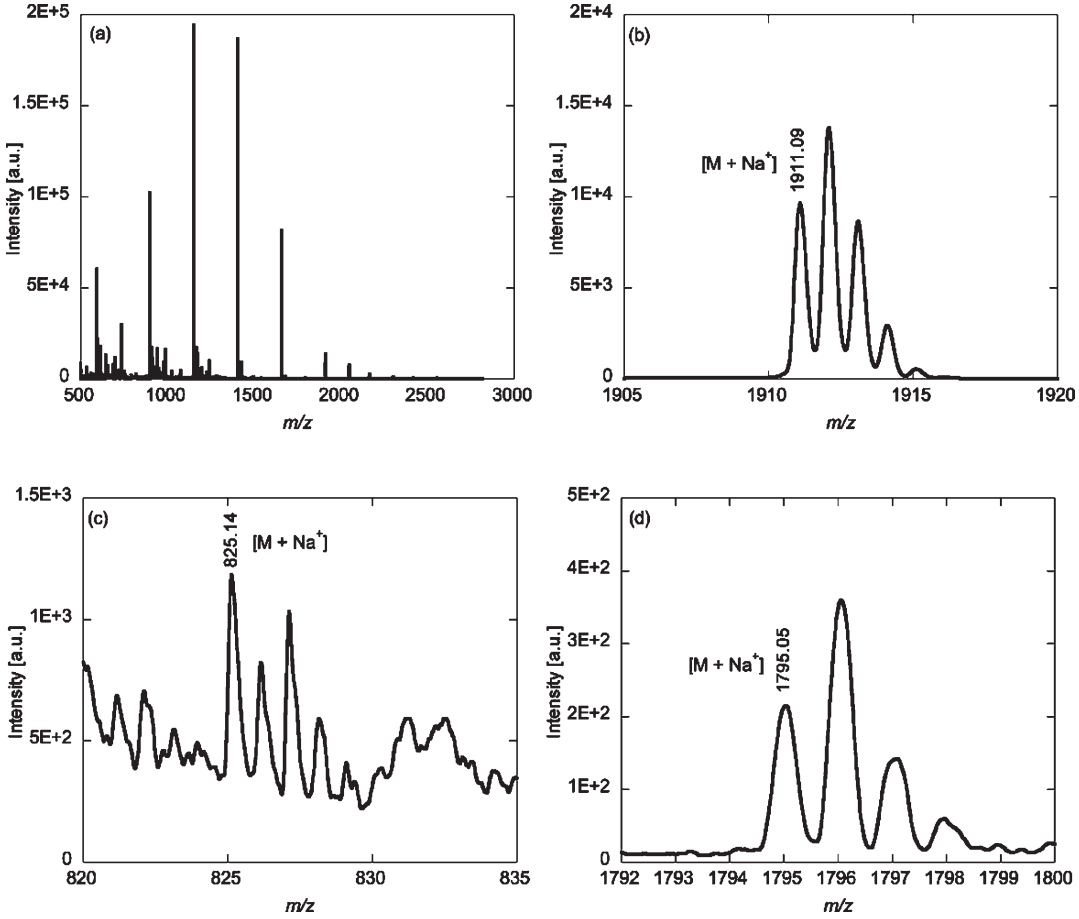


Figure 3. NALDI-TOF MS of GB P(ObLTyr)-2: (a) full spectrum; (b) region of the 7-mer initiated by DAH; (c) region of the 2-mer initiated by AMM; (d) region of the 7-mer initiated by AMM followed by loss of CO_2 by fragmentation.

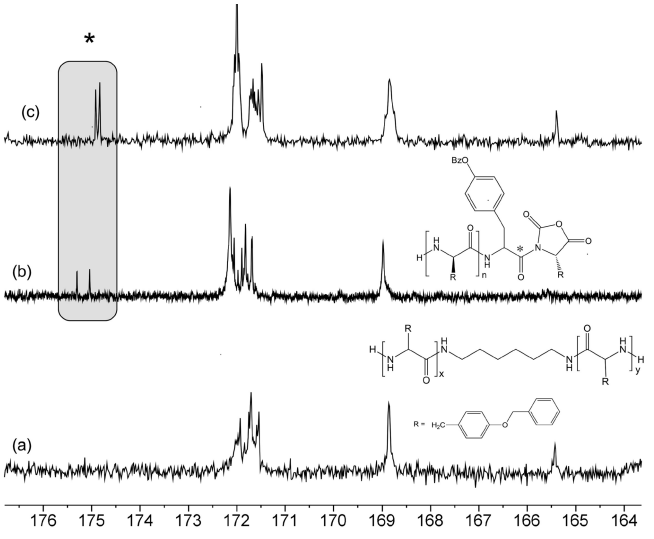


Figure 4. ^{13}C NMR spectra of (a) HV P(ObLTyr), (b) GB P(ObLTyr)-1, and (c) GB P(ObLTyr)-2.

made using high-vacuum techniques. One possible explanation for the presence of products resulting from AMM is the deprotonation of the NCA by dimethylamine (i.e., the source of the odor typically associated with DMF). Unexpectedly, the AMM products were also observed for the polymer prepared in the glovebox with purified DMF (GB P(ObLTyr)-1). This suggests that glovebox techniques do not provide or maintain adequate purity levels to eliminate or suppress the AMM as compared to high-vacuum techniques. It is believed that dimethylamine is formed through decomposition of DMF solvent at the early stages of the polymerization and/or during the ROP process ultimately resulting in deprotonation of the NCA and thus polymerization via AMM.

All polymers were also characterized by ^{13}C NMR spectroscopy to verify the chain-end structure. As shown in Scheme 1, the polymer initiated by the NAM has two primary amine end groups, while that initiated by the AMM mechanism has one amine end and one anhydride end group. The carbons adjacent to the anhydride end group have distinct resonances which can be calculated using the methods of Grant and Paul.[34] There are two regions that demonstrate the presence of the AMM product as shown in Figures 4 and 5. Two peaks are observed at 174.8 and 174.9 ppm in the spectra of the polymers prepared by glovebox techniques (Figure 4b,c). These peaks correspond to the carbonyl adjacent to the AMM end group as shown in Figure 4 (calculated chemical shift[35] 175.2 ppm). Two peaks are observed due to the chiral center adjacent to the carbonyl group. These two peaks are not observed in the spectrum of the polymer prepared by high-vacuum techniques (Figure 4a).

In the second region of interest, two peaks were observed at 59.5 and 54.5 ppm (calculated chemical shifts 64.4 and 54.5 ppm) which correspond to the methylene carbon of the AMM chain end moiety and the carbon β to the nitrogen of the AMM moiety, respectively (Figure 5b,c). These two peaks are not observed in the ^{13}C NMR spectrum of the polymer prepared by high-vacuum techniques (see Figure 5a). The carbon adjacent to a primary amine end-group

cannot be distinguished from the backbone carbons due to their similar chemical surroundings. This is one reason why both MALDI-TOF MS and ^{13}C NMR spectroscopy are essential for complete end-group characterization.

Termination Reactions. While there is no evidence for polymer initiated by AMM when the polymerization is performed by high-vacuum techniques, it is also important to determine if any termination reactions occur. It has been reported that for the amine-initiated polymerization of NCAs there are two main termination routes: reaction with DMF resulting in a formyl end-group and reaction with NCA resulting in a carboxyl end-group (see Scheme 2). According to Giani and co-workers,[16−19] NACE characterization of poly(N_ε-trifluoroacetyl-L-lysine) prepared by Schlenk techniques revealed that 78% of the chain ends were not reactive (carboxylate and formyl end groups) and only 22% of the chains were living (amine end-groups). While Hadjichristidis et al.[12,21] reported the ability to prepare well-defined block copolymers by high-vacuum polymerization of NCAs, they did not investigate the chain-end functionality of the resulting polymers, although the ability to prepare well-defined block copolymers implies a high concentration of living chain ends.

Two different termination products were observed by Giani et al.[17] by NACE, which result from the propagating chain-end reacting with DMF resulting in a formyl-terminated polymer or reaction with C-2 of the NCA resulting in a ureido acid-terminated polymer (see Scheme 2).[20] In addition, the ureido acid-terminated product could also, and more likely, result from reaction of the growing polymer chain with an isocyanocarboxylate, which is derived from the rearrangement of an NCA anion.[16,18] Samples prepared by both high-vacuum techniques as well as by glovebox techniques were analyzed by NALDI-TOF MS to determine the extent of termination. The various termination products and their abbreviations are shown in Scheme 3.

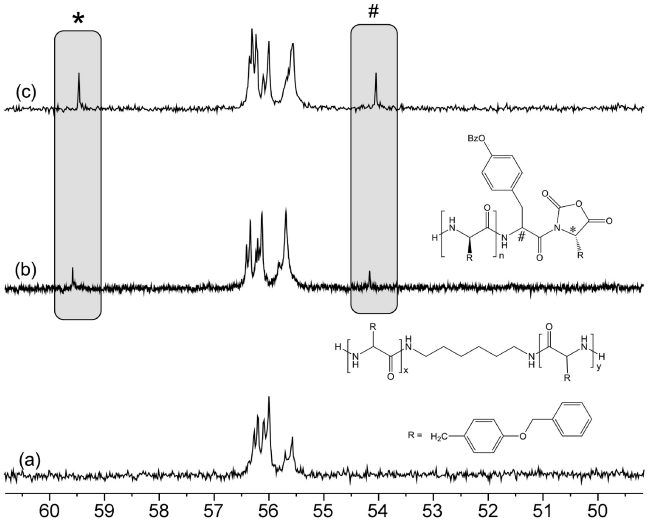


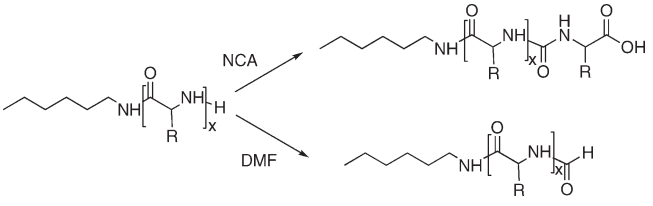
Figure 5. ^{13}C NMR spectra of (a) HV P(ObLTyr), (b) GB P(ObLTyr)-1, and (c) GB P(ObLTyr)-2.

Table 1. Products Observed in Poly(*O*-benzyl-L-tyrosine) Initiated by DAH[a]

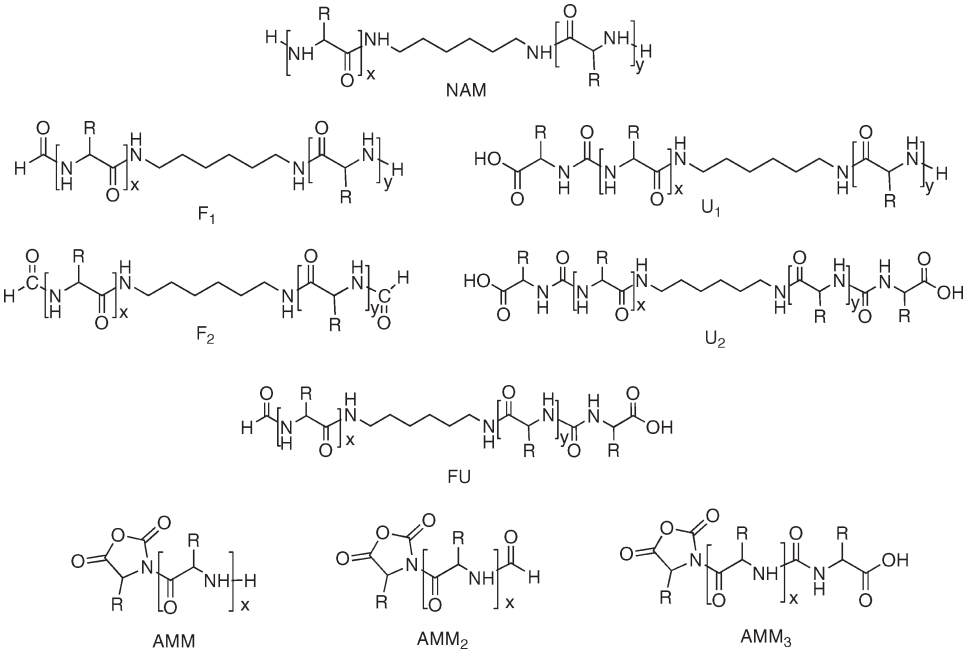
sample	NAM	AMM	AMM_2	AMM_3	F_1	F_2	U_1	U_2	FU
HV	X					X			
GB1	X	X	X	X	X	X			
GB2	X	X	X	X	X	X		X	

[a] HV = high-vacuum technique; GB = glovebox method; 1 = DMF was purified; 2 = DMF used as received; NAM = normal amine mechanism; AMM = activated monomer mechanism; F = formyl end-group; U = ureido acid end-group. These abbreviations match structures shown in Scheme 3.

Scheme 2. Termination of NCA Polymerization via Reaction with NCA or DMF



Scheme 3. Possible Termination Products in the Polymerization of TyrNCA by DAH



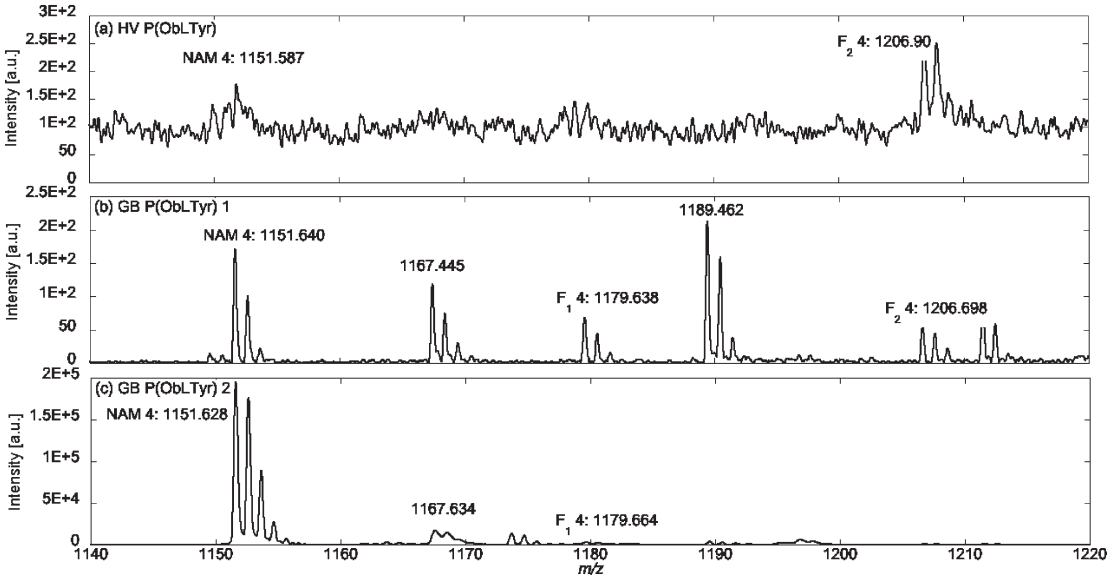


Figure 6. NALDI-TOF MS of termination products for (a) HV P(ObLTyr), (b) GB P(ObLTyr)-1, and (c) GB P(ObLTyr)-2.

Table 1 summarizes the products observed in the NALDI-TOF MS analysis of the DAH-initiated polymerizations of TyrNCA.

Only one termination product is observed in the polymer prepared by high-vacuum techniques, as shown in Figure 6a. This product, F2, results from reaction of both propagating chain ends with DMF, the polymerization cosolvent. The 4-mer, where both propagating chain ends react with DMF, is observed at m/z = 1206.90 and has a calculated monoisotopic mass of 4×253.1103 ($7\times C_{16}H_{15}O_2N$) + 114.1157 ($1\times C_6H_{14}N_2$) + 2×29.0027 (COH) + 22.9898 ($^{23}Na^+$) = 1207.55 Da. Termination due to reaction of the propagating chain end with TyrNCA to form the corresponding ureido acid chain end (U_1 and U_2) is not observed. The peak observed at m/z = 1151.6 is attributed to the 4-mer initiated by DAH (calculated monoisotopic mass 4×253.1103 ($7\times C_{16}H_{15}O_2N$) + 114.1157 ($1 \times C_6H_{14}N_2$) + 2×1.0079 (H) + 22.9898 ($^{23}Na^+$) = 1151.56 Da).

In contrast, samples prepared in the glovebox exhibit multiple termination products resulting from addition to DMF (F_1, F_2) as well as to the NCA (U_1, U_2). The signals for the products resulting from termination with DMF are well-resolved and are observed for multiple degrees of polymers as shown in Figure 6b,c. For example, the calculated monoisotopic mass of the 4-mer of F_1 is 4×253.1103 ($7 \times C_{16}H_{15}O_2N$) + 114.1157 ($1\times C_6H_{14}N_2$) + 1×29.0027 (COH) + 1×1.0079 (H) + 22.9898 ($^{23}Na^+$) = 1179.56 Da. A peak is observed at m/z = 1179.64 as shown in Figure 6b,c. Two other distributions are also observed in GB P(ObLTyr)-1 and GB P(ObLTyr)-2 at m/z = 1167.445 and 1189.462. The peak at m/z = 1167.445 can be attributed to triply charged F_1 13-mer whose calculated monoisotopic mass is 13×253.1103 ($7\times C_{16}H_{15}O_2N$) + 114.1157 ($1\times C_6H_{14}N_2$) + 1×1.0079 (H) + 1×29.003 (COH) + 3×22.9898 ($^{23}Na^+$) = 3503.5292/3 = 1167.84. The peak at m/z = 1189.462 is not identified yet. The ureido acid-terminated product is only observed once in the glovebox prepared material, and the signal is weak and unresolved. In addition, polymer chains initiated by the AMM mechanism which are terminated by reaction with either DMF (AMM_2) or the NCA (AMM_3) are also observed for the 1- and 2-mers. Table 1 shows products that are observed by NALDI-TOF MS in each sample. Signals for the termination products are not observed in the ^{13}C NMR, most likely due to their low concentrations. Unfortunately, NALDI-TOF MS cannot be used for quantification of the various products identified due to the unknown variability in the ionization of the molecules.

Finally, an intramolecular termination step that is commonly observed with poly(γ-*O*-alkyl-L-glutamates) is the cyclization of the chain-end where the primary amine undergoes a backbiting reaction with the pendant alkyl ester, resulting in a terminal lactam.[3] TyrNCA does not appear to be susceptible to this same termination step as the protected pendant group is an ether rather than an ester, and thus no electrophilic centers are present to facilitate such a backbiting reaction. Moreover, there are no peaks observed by any of the spectroscopic techniques (MALDI-TOF MS, NALDI-TOF MS, ^{13}C NMR) employed in this study, which are characteristic of a terminal lactam.

Conclusions

This is the first study to investigate the chain-end functionality of polypeptides prepared by high-vacuum techniques. These results demonstrate that the polymerization of TyrNCA by high-vacuum techniques proceeds exclusively by the normal amine mechanism with minimal termination. Analysis by NALDI-TOF MS shows only one major distribution that is attributed to the primary amine-terminated polypeptide; a small distribution due to termination by reaction with the polymerization solvent, DMF, is also observed. In contrast, polypeptides prepared in the glovebox proceed by both the normal amine mechanism and the activated monomer mechanism, as demonstrated by NALDI-TOF MS measurements. Multiple termination products are observed, including formyl- and ureido acid-terminated chain ends, and these structures are confirmed by ^{13}C NMR spectrometry. Thus, the synthesis of well-defined polypeptides can be achieved with the use of high-vacuum techniques. The materials synthesized by this method more closely represent natural polypeptides because the amine end-group is retained, which also provides a reactive moiety for subsequent functionalization, including chain extension.

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