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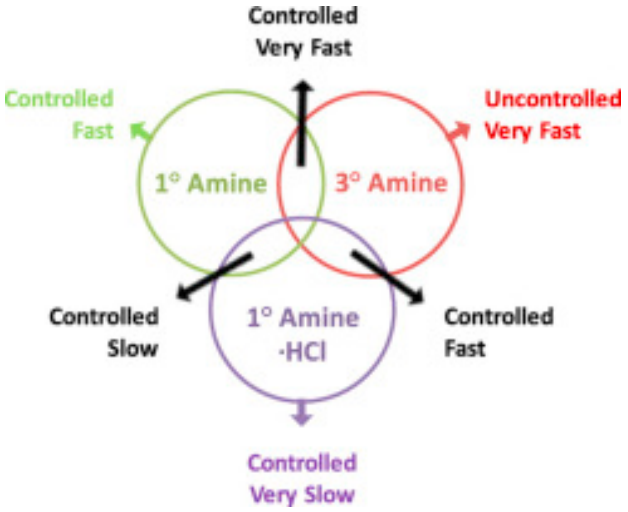

Research highlights

· The mechanism of ring-opening polymerization of amino acid NCA is re-examined.
· Normal amine and activated monomer mechanisms are shown to co-exist.
· Fast and controlled polymerizations for primary/tertiary amine mixed initiators.
· Level of control depends on the ratio of primary to tertiary amine.

Controlled ring-opening polymerization of α-amino acid *N*-carboxyanhydrides in the presence of tertiary amines

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Abstract

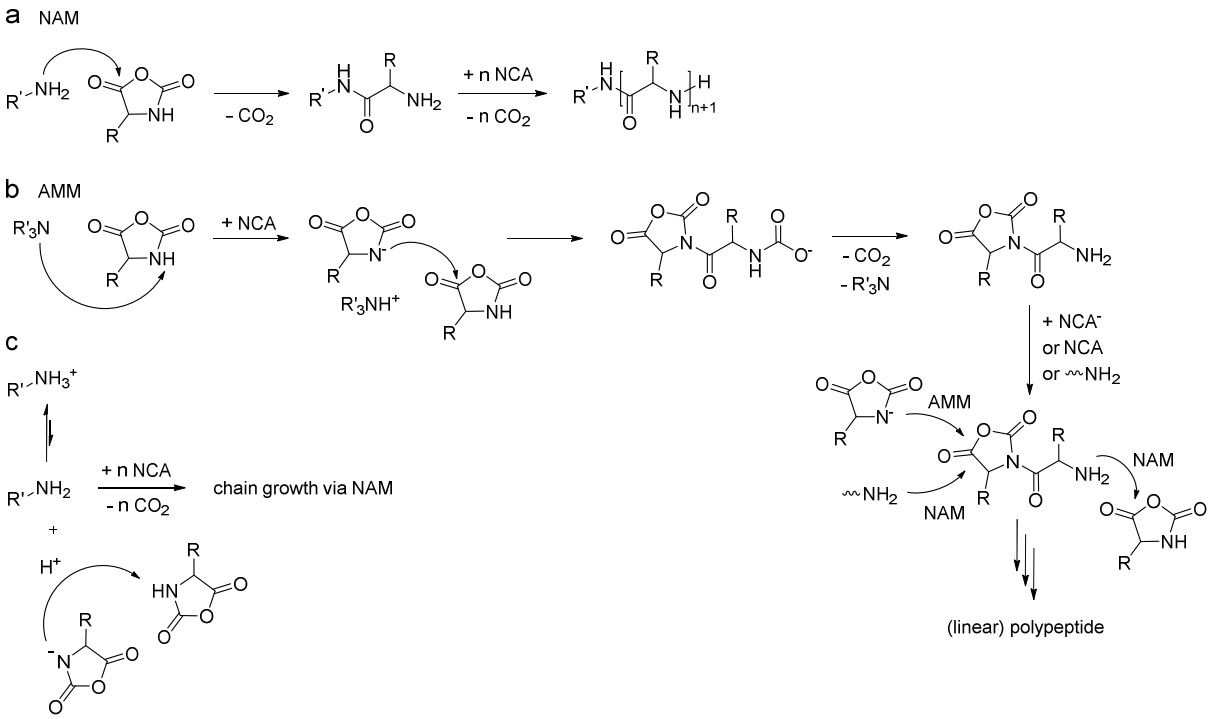
The mechanism of the primary ammonium/tertiary amine-mediated ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) was investigated. Kinetic analyses revealed that the normal amine mechanism (NAM) together with a dormant-active chain end equilibrium were responsible for the controlled nature of this polymerization pathway, but that the polymerization also proceeded via the activated monomer mechanism (AMM). Mixtures of primary amines (1 equiv) and tertiary amines (0 to 1.5 equiv) were therefore tested to confirm the co-existence of the NAM and AMM and determine the limits for a controlled polymerization. For tertiary amine molar fractions smaller than 0.8 equiv, the reaction times were greatly reduced (compared to primary amine-initiated polymerization) without compromising the control of the reaction. Hence, the polymerization of NCA can proceed in a controlled manner even when the AMM contributes to the overall chain growth mechanism.

Introduction

The controlled nature of a polymerization reaction is a key to the synthesis of well-defined polymers, which are ubiquitous in the fields of self-assembly and biomimicry, notably for the preparation of biomaterials and medical materials [1-5]. Polypeptides are polymers of great interest for such applications, not only because they can be designed to be biocompatible and biodegradable, but also because they can be synthesized in a controlled manner by ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCA) [6-9]. Since the late 1940s, NCA polymerization has been the most common technique used for large-scale preparation of high molar mass polypeptides [6].

NCA polymerizations are traditionally initiated or catalyzed using nucleophiles or bases, respectively, the most common being amines and alcohols [6] as well as thiols [10]. Depending on the type of initiator and reaction conditions, the ROP of NCA is known to mainly proceed *via* two different pathways, namely the 'normal amine' mechanism (NAM) and the 'activated monomer' mechanism (AMM) [6, 11]. The NAM is a nucleophilic ring-opening chain growth process (Scheme 1a) and yields well-defined polypeptides. The AMM takes place following the deprotonation of an NCA and hence the generation of a nucleophilic NCA anion. Acting as an initiator, the NCA anion adds to another NCA, thereby yielding a dimer with both a reactive *N*-acylated NCA α-end and primary amine ω-end. As such, this propagating species can further react with both deprotonated NCA and NCA or even undergo condensation reactions with primary amine chain ends (Scheme 1b) to yield linear polypeptides with high molar mass and dispersity. Therefore, the NAM is favored by nucleophilic initiators that are less basic than nucleophilic, such as aliphatic primary amines, whereas the AMM is favored by basic catalysts

that are more basic than nucleophilic, such as sterically hindered secondary amines and most tertiary amines. However, the system can switch back and forth between NAM and AMM [11].



Scheme 1. (a) Normal amine mechanism (NAM), (b) activated monomer mechanism (AMM), and (c) proposed mechanism for the ammonium-mediated ring-opening polymerization of NCA.

In an effort to eliminate side reactions, notably the AMM, and to achieve better control in the polymerization of NCA, a number of methods have been developed through the use of transition metal catalysts [12], organocatalytic systems [13, 14], hexamethylsilazane [15] and ammonium salt [16] as initiators or by working under highly pure conditions [17] or at lower temperature [18]. The ammonium-mediated ROP of Z-L-lysine-NCA, initiated by primary amine hydrochlorides, was proposed to proceed *via* a pathway involving an equilibrium between dormant and active chain ends, with the active species propagating *via* the NAM, thereby producing polypeptides with predictable and narrowly distributed molar masses (Scheme 1c) [16]. This method has been shown to be also successful for other initiators (*e.g.*, pyrene-, *n*-

butyl-, polymer-ammonium) and counterions (*e.g.*, BF_4^-, CF_3COO^-, Cl^-) [19-22]. It was further refined by using mixtures of ammonium chlorides and their corresponding amines, allowing to reduce the reaction times of otherwise slow ammonium-mediated ROP of γ-benzyl-L-glutamate-NCA (BLG-NCA) and β-benzyl-L-aspartate-NCA [23].

More recently, it was shown that tertiary amines could be used to catalyze the ammonium chloride-initiated ROP of NCA without compromising the control of the polymerization [24]. This result was surprising as tertiary amines used alone typically promote the AMM in ROP of NCA. It was proposed that tertiary amines being more basic than primary amine, the protons were preferentially drawn from the primary ammonium chloride species by the tertiary amines, thereby freeing primary amines and inducing the NAM. As evidence of this amine-ammonium equilibrium and of the inhibiting role of protons, a polymerization initiated by a primary ammonium chloride and tertiary amine mixture was paused after a few hours by adjusting the total amine/HCl ratio to unity, and was later resumed by the addition of tertiary amines. Moreover, this so-called primary ammonium/tertiary amine-mediated polymerization remained controlled even when a slight excess of tertiary amine (1.1 equiv) relative to primary ammonium chloride (1 equiv) was used. This result, therefore, suggests that both the NAM and the AMM contributed towards the overall propagation mechanism of primary ammonium/tertiary amine-mediated ROP of NCA. The present study sought to further investigate this polymerization mechanism and its kinetics. Notably, initiator mixtures of primary and tertiary amines were used to induce both NAM and AMM and investigate the effect and limits of the co-existence of both pathways on the controlled nature of the polymerization.

Experimental section

Materials. Acetic anhydride (Ac_2O) (99%), benzylamine ($BnNH_2$) (99%), γ-benzyl-L-glutamate (BLG) (≥99%), *N*,*N*-dimethylformamide (DMF) (99.8%, anhydrous), 1,4-dioxane (99.8%), maleic anhydride (99%), 1-hexylamine ($HexNH_2$) (99%), triphosgene (98%), and deuterated chloroform ($CDCl_3$) (99.96 atom% D) were purchased from Sigma-Aldrich. Tetrahydrofuran (THF) (99.5%, anhydrous), triethylamine (TEA) (99%), and deuterated 1,1,1-trifluoroacetic acid (TFA-d) (99.5 atom% D) were purchased from Acros Organics. BLG-NCA and benzylamine hydrochloride ($BnNH_2 \cdot HCl$) were prepared according to earlier reported procedures [24].

Polymerizations. Typically, BLG-NCA (100 equiv) were placed in a flame-dried flask and dried under high vacuum for 1 hour. The appropriate amount of dry DMF was added under inert atmosphere to reach a concentration of 100 $g \cdot L^{-1}$, unless mentioned otherwise. The mixture was cooled down in ice for 20 min and the initiator/catalyst solution (in dry DMF) was added. This solution contained either $BnNH_2$ (1 equiv), $BnNH_2 \cdot HCl$ (1 equiv), TEA (0.5 equiv), $BnNH_2 \cdot HCl$ (1 equiv) and TEA (between 0.5 and 1.5 equiv), or $BnNH_2$ (1 equiv) and TEA (between 0.5 and 1.5 equiv). The reaction medium was then placed under a slight overpressure of dry nitrogen and maintained at room temperature. For the kinetic study, regular sampling was performed using dry nitrogen-purged syringes. For the SEC characterization, samples were terminated by addition of maleic anhydride (> 50 equiv), stirred for 20 min and then mixed with the SEC eluent prior to injection on the SEC column. For the NMR characterization, samples were terminated by addition of maleic anhydride (> 50 equiv), stirred for 20 min and then precipitated in cold methanol. The product was collected by centrifugation (9000 rpm, 5 min), re-precipitated in diethyl ether, washed, dried under high vacuum, and freeze-dried from 1,4-dioxane.

Analytical instrumentation and methods. ^{1}H NMR spectra were recorded on a Bruker Avance III 600 MHz Spectrometer at room temperature; the number of scans was at least 32. Size exclusion chromatography (SEC) with simultaneous UV and RI detection was performed with *N*-methyl-2-pyrrolidone (NMP + 0.5 wt% LiBr) as the eluent at a flow rate of 0.8 mL·min^{-1} at 70 °C; the stationary phase was a set of two 300 x 0.8 mm^2 PSS-GRAM columns (7 μm particle size, 100 and 1000 Å porosity). Poly(methyl methacrylate) (PMMA) standards (PSS GmBH, Mainz, Germany) were used for calibration. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was performed on a Bruker Vertex 70 fitted with PLATINUM ATR. Liquid samples were placed directly on the ATR diamond under a mixture of dry air and nitrogen. The spectra were acquired and processed with OPUS; the number of scans was 16, the built-in atmospheric correction function was turned on, and the background (*i.e.*, solvent) was automatically subtracted. The spectra were then processed as follows: portions of interest (*e.g.*, 1758-1815 cm^{-1}) of the spectra were isolated ('cut' function), baseline corrected ('concave rubberband correction', 1 iteration), and fitted with Gaussian functions ('curve fit' function).

The NCA monomer conversion was determined by ATR-FTIR spectroscopy, taking advantage of the fact that the absorbance peaks of the carbonyl groups of BLG-NCA ($\tilde{\nu}$ ($\nu_{(C\text{-})C=O}$) = 1850-1855 cm^{-1} and $\tilde{\nu}$ ($\nu_{(N\text{-})C=O}$) = 1785-1790 cm^{-1}) do not overlap with the absorbance peaks of the polypeptide ($\tilde{\nu}$ ($\nu_{C=O\ ester}$) ~ 1730 cm^{-1} and $\tilde{\nu}$ ($\nu_{Amide\ I}$) ~ 1650 cm^{-1}). The monomer concentration was extracted from the area under the largest C=O peak (1785-1790 cm^{-1}) using a calibration curve as previously reported [24].

Results and discussion

In an 'ideal' case of living/controlled ROP of NCA, *i.e.*, either *via* NAM only (Scheme 1a) or dormant-active amine-ammonium equilibrium (Scheme 1c), and under the assumption of absence of side reactions, the polymerization kinetics can be resolved and simplified to a pseudo first-order kinetics, *i.e.*, $-\ln(1-x_p) = k_{app} \cdot t$, x_p: monomer conversion, k_{app}: apparent rate constant of propagation, t: time. Any deviation from linearity would imply that the initial assumption of an ideal mechanism is incorrect or incomplete, in which case 'non-ideal' models ought to be considered, such as a NAM-AMM co-existence model.

The first-order time-conversion plots of a series of ROP of BLG-NCA at room temperature initiated by benzylamine hydrochloride ($BnNH_2 \cdot HCl$), benzylamine ($BnNH_2$), mixtures of $BnNH_2 \cdot HCl$ and triethylamine (TEA), and TEA are shown in Figure 1a; monomer conversions were determined by ATR-FTIR spectroscopy [24]. All plots – with the exception of the exceedingly slow $BnNH_2 \cdot HCl$-initiated polymerization – exhibited a concave curvature at low conversions before 'straightening up' to reveal a more linear behavior (similar to the first-order plots observed for the anionic polymerization of methyl methacrylate in the presence of aluminium alkyls in toluene [25]). This is indicative of a fast conversion at an early propagation stage, followed by a NAM-controlled propagation (combined with a dormant-active chain end equilibrium in the case of ammonium salt initiators). This phenomenon was observed not only for primary ammonium/tertiary amine-mediated but also for the more traditional primary amine-initiated polymerization.

Most importantly, this result indicates that none of the studied polymerizations proceeded *via* a NAM-only or NAM and dormant-active mechanism, thereby supporting a 'non-ideal' propagation model. As pointed out by Deming, the abundance of potential reactions in ROP of

NCA makes it difficult to achieve a living polymerization system where only chain propagation occurs, even when using only primary amines as initiators [9]. In other words, first-order time-conversion plots can be expected to deviate from linearity even when the initiators are primary amines, which indeed has already been reported in other studies [20, 26]. Nevertheless, both primary ammonium/tertiary amine- and primary amine-initiated ROP of NCA yielded polymers with predictable molar mass (^{1}H NMR) and narrow molar mass distribution (SEC, Figure 1b) together with well-defined end groups (α-/ω-end group ratio ≈ 1, Figure 2), pointing towards the NAM as the prevailing chain growth mechanism. It is worth pointing out that termination by back-biting, *i.e.*, formation of pyroglutamate chain ends, can be mainly excluded as this should have been recognized by an α-/ω-end group ratio greater than unity. Also, the absolute number-average molar masses (M_n), as determined by ^{1}H NMR end group analysis, were about twice as large as the apparent M_n values from SEC analysis based on PMMA calibration; however the dispersity values ($Đ = M_w/M_n$; ratio of weight- to number average molar mass) ought to be rather reliable.

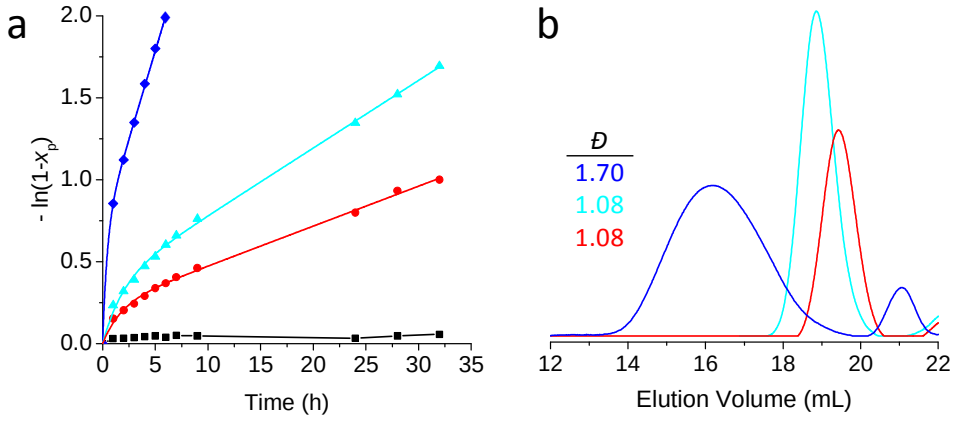


Figure 1. (a) First-order time conversion plots for the polymerizations of BLG-NCA (100 equiv) in DMF at room temperature initiated by $BnNH_2$·HCl (1 equiv, black squares), $BnNH_2$·HCl/TEA (1:0.5 equiv, red circles), $BnNH_2$ (1 equiv, cyan triangles), and TEA (0.5 equiv, blue diamonds); lines in-between data points are added to guide the eye. (b) SEC-RI traces of polyBLG obtained after initiation by $BnNH_2$·HCl/TEA (1:0.5 equiv, red line), $BnNH_2$ (1 equiv, cyan line), and TEA (0.5 equiv, blue line) after a reaction time of ~24 h.

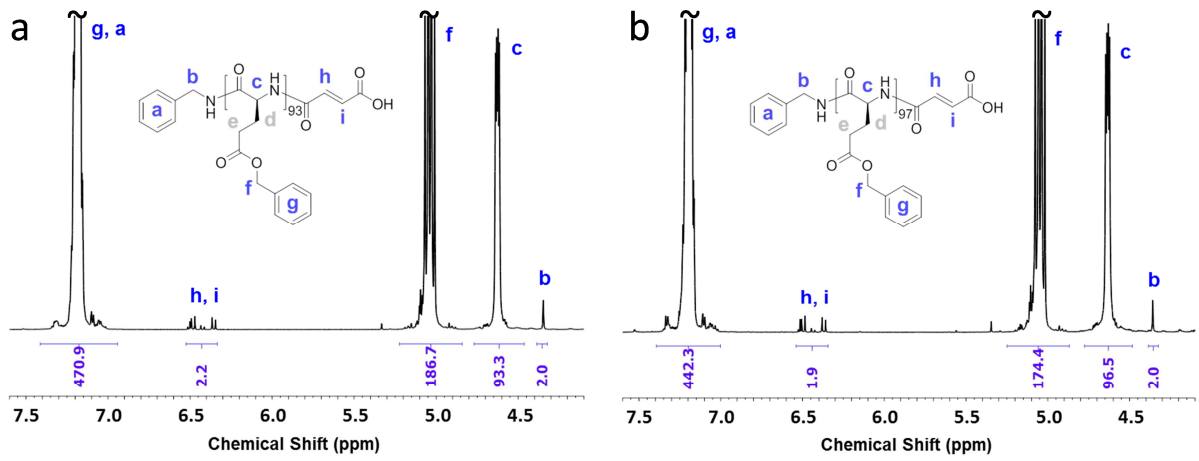


Figure 2. ^{1}H NMR spectra (4.1-7.6 ppm region, 600 MHz, TFA-*d*) of polyBLG isolated from the polymerizations of BLG-NCA (100 equiv) in DMF at room temperature and initiated by (a) $BnNH_2$·HCl/TEA (1:0.5 equiv), and (b) $BnNH_2$ (1 equiv), after a reaction time of 72 h; the ω-end groups arise from the termination step with maleic anhydride.

In the following, we were aiming at to identify the origin of the non-linear first-order time-conversion plots and if also the AMM contributed to chain propagation. The tertiary amine-catalyzed ROP of NCA is known to proceed *via* the AMM, a mechanism that enables the rapid achievement of high conversions and broad molar mass distributions, principally due to the regeneration of the tertiary amine and the presence of two active sites on the propagating chains (*i.e.*, *N*-acylated NCA at the α-end, and primary amine at the ω-end, see Scheme 1b) [27]. This is supported by the relatively large polymerization rates of TEA-initiated ROP of BLG-NCA (Figure 1a) and the large dispersity of the corresponding polyBLG (Figure 1b). In light of these considerations, it could be rationally postulated that for primary ammonium/tertiary amine- and primary amine-initiated ROP of NCA, both NAM and AMM co-exist, with the AMM predominating in the early stages of propagations, as indicated by initially rapid conversions. To provide further evidence to this NAM-AMM co-existence model, initiator mixtures of primary and tertiary amines were investigated.

The first-order time-conversion plots of a polymerization series of BLG-NCA initiated by mixtures of $BnNH_2$ (1 equiv) and TEA (0 to 1.5 equiv) are shown in Figure 3a. As the molar fraction of TEA increased, the time conversion plots became gradually more ‘concave’ and the molar mass distributions of polyBLG broadened (Figure 3b). The control over the polymerization was lost when the fraction of TEA exceeded 0.8 equiv relative to $BnNH_2$, as indicated by dispersity values greater than 1.5. This result is coherent with the observation, in an earlier study, that primary ammonium chloride/TEA-mediated ROP of NCA became uncontrolled when the TEA fraction exceeded 1.5 equiv relative to the ammonium species [24]. Moreover, the ROP of BLG-NCA initiated with $BnNH_2$/TEA (1:0.5 equiv) exhibited very similar time-conversion plots and molar mass distributions as when initiated with $BnNH_2$·HCl/TEA (1:1.5 equiv) (Figure 4).

These results further consolidate the hypothesis by which NAM and AMM co-exist during the chain growth of both primary ammonium/tertiary amine- and primary amine-initiated ROP, and that the NAM is the prevailing mechanism, ensuring the controlled nature of the polymerization, as long as the tertiary amine remains under about 1.5 equiv and 0.5 equiv relative to primary ammonium salt and primary amine, respectively. The overall predominance of the NAM in these polymerizations was further supported by the quantitative incorporation of $BnNH_2$ initiators at the α-end of growing chains (Figure 5). It is further worth pointing out that a NAM-AMM mixed mechanism would not have affected the polypeptide chain topology and produced exclusively linear polypeptide chains (Scheme 1b).

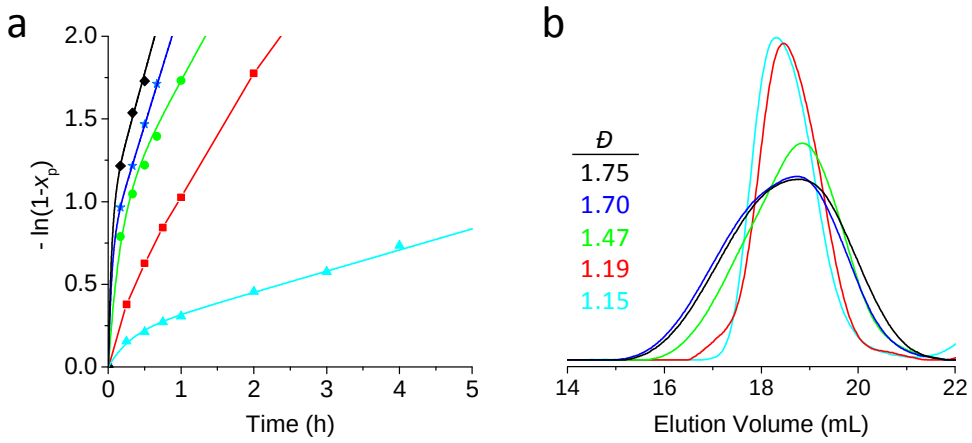


Figure 3. (a) First-order time-conversion plots for the polymerizations of BLG-NCA (100 equiv) in DMF at room temperature initiated by $BnNH_2$/TEA = 1:0 equiv (cyan triangles), 1:0.5 (red squares), 1:0.8 (green circles), 1:1.1 (blue stars), and 1:1.5 (black diamonds); lines in-between data points are added to guide the eye. (b) SEC-RI traces of polyBLG obtained after initiation by $BnNH_2$/TEA = 1:0 equiv (cyan line), 1:0.5 (magenta line), 1:0.8 (green line), 1:1.1 (blue line), and 1:1.5 (navy blue line) after a reaction time of ~24 h.

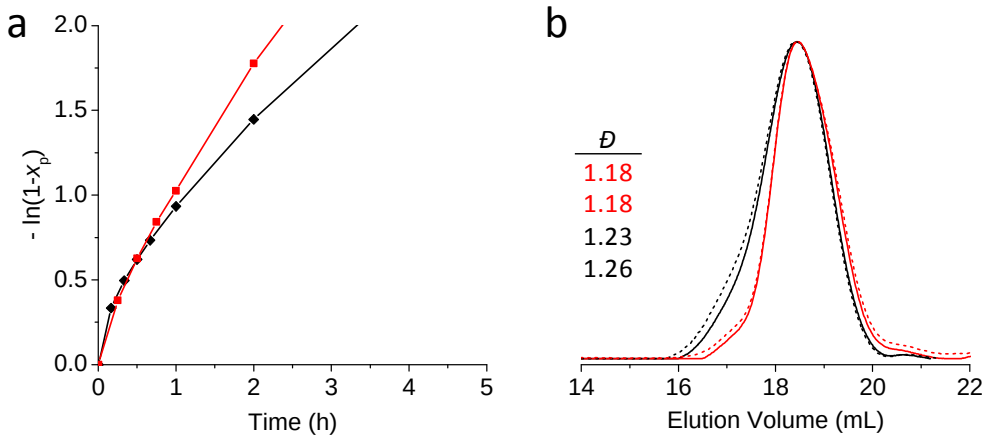


Figure 4. (a) First-order time-conversion plots for the polymerizations of BLG-NCA (100 equiv) in DMF at room temperature initiated by $BnNH_2$/TEA = 1:0.5 equiv (red squares) and $BnNH_2$·HCl/TEA = 1:1.5 equiv (black diamonds); lines in-between data points are added to guide the eye. (b) SEC-RI traces of polyBLG obtained after initiation $BnNH_2$/TEA = 1:0.5 equiv (red lines) and $BnNH_2$·HCl/TEA = 1:1.5 equiv (black lines) after a reaction time of ~24 h (solid lines) and ~50 h (dashed lines).

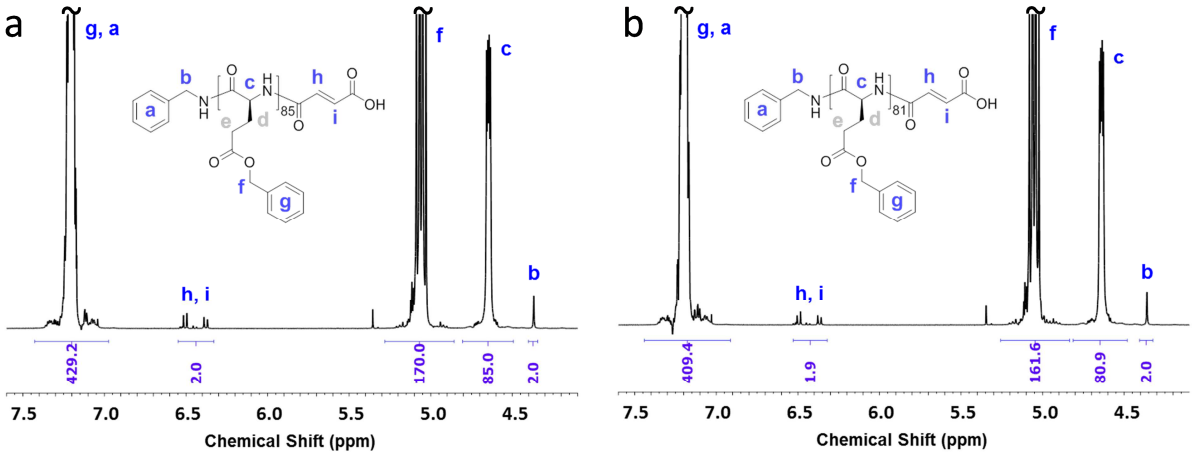


Figure 5. ^{1}H-NMR spectra (4.1-7.6 ppm region, 600 MHz, TFA-*d*) of polyBLG isolated from the polymerizations of BLG-NCA (100 equiv) in DMF at room temperature and initiated by (a) $BnNH_2$ (1 equiv), and (b) $BnNH_2$/TEA (1:0.5 equiv), after a reaction time of 32 h; the ω-end groups arise from the termination step with maleic anhydride.

Interestingly, the present study somewhat corresponds to a kind of control experiment in the context of studies on accelerated amine mechanism through monomer activation (AAMMA), which has been proposed when polymerizations were conducted with initiators comprising of both primary and secondary/tertiary amine functional groups [13, 28]. In such cases the polymerizations followed first-order kinetics and produced narrowly dispersed polymers and block copolymers, involving the activation of NCA monomers by the secondary/tertiary amines present at the α-end (where the initiator is incorporated) of each polymer chain; the possibility that the NAM and the AMM could co-exist and lead to a controlled polymerization was ruled out. However, in the present study we showed that well-defined polypeptides with low molar mass distributions were obtained even when both the NAM and AMM were shown to both contribute towards the propagation of chains (Figures 3 and 4). The advantage of using a mixture of primary and tertiary amines is that their ratios can be precisely set, which allowed, in the present study, for the observation of a gradual and smooth transition from a controlled to an uncontrolled polymerization (see above). This observation points towards a NAM-AMM co-existence mechanism, where the relative preponderance of one or the other mechanism can be adjusted by the primary/tertiary amine ratio.

As highlighted in the present study, the NAM-AMM co-existence mechanism for the ROP of NCA can be induced with either (i) primary ammonium salts/tertiary amines, (ii) primary amines/tertiary amines, or (iii) primary amines only. An earlier study also confirmed that, in the presence of protons (from the ammonium species), the dormant-active chain end equilibrium was also involved (Scheme 1c) [24]. The first-order time-conversion plots of all monitored polymerizations (Figures 1 and 3) suggest that the AMM plays a greater role in the early stage of the polymerization, as well as whenever greater molar fractions of tertiary amines are used

relative to primary ammonium or amine species. This hypothesis is supported by a study on the AMM conducted by Kricheldorf [29]. Like all prototropic reactions of acidic protons, the AMM initiation step, where a proton of the amino group of an NCA monomer is abstracted by a strong base, is rapid. Notably, it is faster than the NAM initiation step, which consists of a nucleophilic attack of a primary amine onto an NCA monomer. This explains the rapid conversion observed early in the propagation step. Moreover, an *N*-acylated NCA species (generated by the AMM at the α-ends of growing chains) is more electrophilic than the corresponding NCA monomer itself, which means that is will be preferentially consumed over NCA monomers by both primary amines (*e.g.*, initiator and ω-ends of growing chains) and anions (*e.g.*, NCA anions and carbamate ions). This explains the predominance of the NAM in the later stages of the propagation.

In order to understand how other parameters, such as temperature and monomer concentration affect the primary ammonium/tertiary amine-mediated ROP of BLG-NCA, a series polymerizations initiated by $BnNH_2 \cdot HCl$/TEA initiator/catalyst mixtures were examined. The $BnNH_2 \cdot HCl$/TEA ratios were kept within the pre-established range to maintain the polymerization controlled, *i.e.*, 1:*x* with $x < 1.5$ (all polymers isolated after 24 h exhibited narrow molar mass distributions, $Đ$ = 1.08-1.18, thereby confirming the controlled regime). A near-linear first-order plot was obtained when a lower concentration of NCA was used, 50 $g \cdot L^{-1}$ instead of 100 $g \cdot L^{-1}$ (as used in the rest of this study), whereas the plot further deviated from linearity at 200 $g \cdot L^{-1}$ (Figure 6a). Evidently, using a lower NCA concentration may lower the incidence of side reactions and/or the occurrence of the AMM. Indeed, the AMM is likely to be associated with reaction orders greater than 1 with respect to NCA monomers (Scheme 1b), and would thus be more strongly affected by a change of NCA concentration than the NAM and the

dormant-active chain end equilibrium, which are first-order reactions with respect to NCA monomers (Scheme 1a).

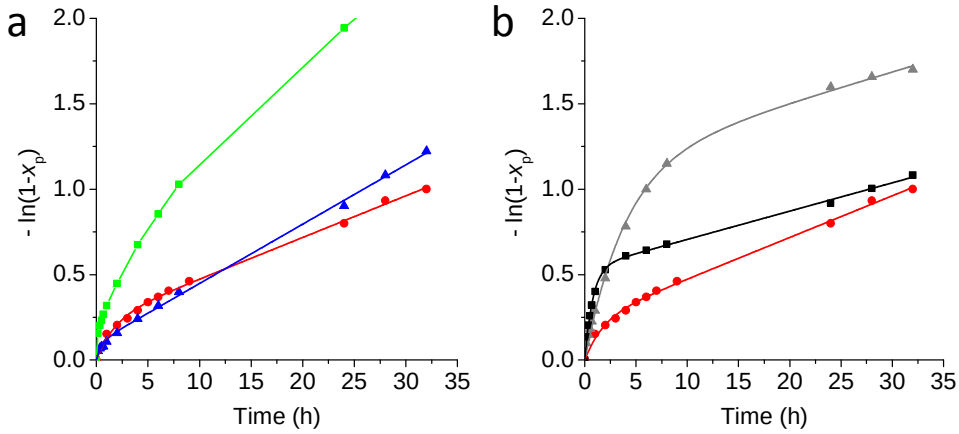


Figure 6. First-order time conversion plots for the polymerizations of BLG-NCA (100 equiv) in DMF initiated by $BnNH_2$·HCl/TEA (1:0.5 equiv): (a) $[BLG\text{-}NCA]_0$ = 50 g·L^{-1} (blue triangles), 100 g·L^{-1} (red circles), and 200 g·L^{-1} (green squares), room temperature; (b) $[BLG\text{-}NCA]_0$ = 100 g·L^{-1} at room temperature (red circles), 50 °C (grey triangles), and 80 °C (black squares); lines in-between data points are added to guide the eye.

Higher temperatures led to first-order plots that exhibited a larger slope in the early propagation stage (2 to 8 h) followed by a more pronounced change in slope, compared to the room temperature polymerization (Figure 6b). Such changes are in agreement with the proposed polymerization mechanism in which the NAM, the AMM and a dormant-active species equilibrium co-exist. Indeed, their equilibrium constants and reaction rates are likely to be affected by the temperature in different ways, resulting in variable time-conversion behaviors, an accurate prediction of which would require a complete kinetic description of the mechanisms involved.

Conclusion

The kinetics and mechanism of the previously established primary ammonium/tertiary amine-mediated ROP of BLG-NCA was investigated. The polymerization was controlled and yielded polypeptides with predictable molar masses and narrow molar mass distributions, however it did not follow a simple first-order kinetics which indicated that the NAM and the dormant-active species mechanism could not be solely responsible for the chain growth. It appeared that the rapid monomer conversion in the early stages of the polymerization was enabled by the AMM, while the NAM prevailed for the remaining of the propagation, thereby maintaining the control of the polymerization.

In order to test this hypothesis, a series of polymerizations was initiated with mixtures of primary and tertiary amines. Primary amine/tertiary amine-initiated ROP of NCA remained controlled even for initiator mixtures containing up to 0.8 equiv of tertiary amine relative to the primary amine. Moreover, there was no sharp increase, rather a gradual increase of the dispersity with increasing tertiary amine fractions. This result highlighted the fact that the level of control of the ROP of NCA can be finely tuned by the composition of the initiator mixture, more precisely by the primary amine/tertiary amine molar ratio, through a fine balance between the NAM and the AMM. This outcome hence revealed a notion of gradual decrease or increase of control, rather than a 'black or white' model. As such, it can be concluded that the control of primary ammonium/tertiary amine-mediated ROP of NCA is directly related to the ratio of primary amine/tertiary amine/Brønsted-Lowry acid (*e.g.*, hydrochloride), with the tertiary amine essentially acting as a catalyst.

Future work shall focus on the identification of the actual reaction kinetics of the proposed AMM-NAM-mixed mechanism, and more precisely determine the effect of temperature,

counter-ion, NCA concentration, and solvent. It is also worth stressing that the AMM pathway is known to favor stereospecificity (*i.e.*, tacticity resulting from the copolymerization of D and L monomers) [30] as well as to be affected by the nucleophilicity of *N*-acylated NCA chain ends (which may vary with the nature of the NCA) [29]. As such, the copolymerization of NCA monomers by primary amine/tertiary amine-initiated ROP of NCA ought to be thoroughly investigated for different types of comonomers, particularly in terms of the resulting polypeptide compositions and monomeric unit distributions.

Acknowledgment

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