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Accelerated and Controlled Polymerization of *N*-Carboxyanhydrides in the Presence of Tertiary Amines with Minimized Activated Monomer Mechanism

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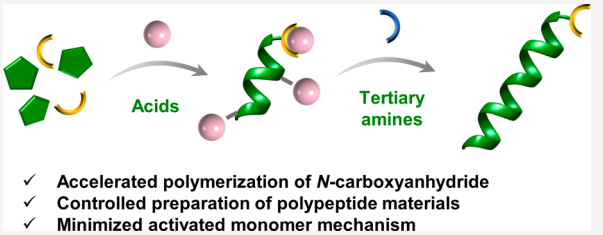
ABSTRACT: Synthetic polypeptides, mainly prepared through the ring-opening polymerization (ROP) of amino acid *N*-carboxyanhydrides (NCAs), have garnered significant interest for biomedical and material applications. Tertiary amines, such as triethylamine (TEA), typically initiate the polymerization of NCAs through the activated monomer mechanism (AMM), which proceeds in a fast but uncontrolled manner, and thus are no longer used for polypeptide synthesis. In this study, we demonstrate that the addition of acetic acid (AcOH) effectively modulates the reactivity of tertiary amines to suppress the AMM initiation while retaining its accelerating role, enabling rapid and controlled preparation of polypeptide materials. With the sequential addition of AcOH and TEA, well-defined polypeptides are synthesized with predictable molecular weights and narrow dispersity within minutes. Detailed mechanistic studies suggest that the partial protonation of TEA by AcOH facilitates the monomer activation while minimizing the NCA deprotonation. This work provides new insights into the role of tertiary amines in ROP of NCA, offering a simple and efficient strategy for the scalable production of high-quality polypeptide materials.



INTRODUCTION

Synthetic polypeptides, serving as structural and functional analogues of natural proteins, have attracted considerable attention for applications in drug delivery, tissue engineering, and antimicrobial materials.[1−11] Among various synthetic strategies, the ring-opening polymerization (ROP) of amino acid *N*-carboxyanhydrides (NCAs) represents a powerful method for preparing polypeptides with tunable structures. Depending on the initiation step and the chain-end of the propagating species, conventional ROP of NCA proceeds through two distinct pathways: slow but controlled "normal amine" mechanism (NAM) and fast but uncontrolled "activated monomer" mechanism (AMM) (Scheme 1).[12−15] While primary amines are widely used as NAM initiators, tertiary amines are believed to mainly initiate polymerization through AMM.

Due to the requirement to prepare well-defined polypeptide materials with predictable molecular weights (MWs) and narrow dispersity, significant efforts have been devoted to improving the NAM polymerization systems in recent decades. Notably, recent advances in accelerated polymerization facilitated the rapid synthesis of polypeptides with minimized side reactions.[16−23] The cooperative covalent polymerization (CCP) in low-polarity chlorinated solvents, for instance, enabled the NCA polymerization even in the presence of a water phase.[16,18,24−31] Meanwhile, few systems utilize AMM initiators these days despite their fast kinetics.[32] Considering the simple structure, low costs, and high reactivity of tertiary amines such as triethylamine (TEA), it would be interesting to retain their role as kinetic accelerators while suppressing their initiations that led to uncontrolled polypeptide growth. Previous reports suggested the suppressed initiation of tertiary amines with specially designed initiator structures.[33−35] With recent studies on various catalysts for NCA polymerization, especially protic acids, we reasoned that it is possible to fine-tune the reactivity of tertiary amines to facilitate the desired rate acceleration while minimizing the undesired AMM initiation.

Herein, we report the fast and living polymerization of NCA in the presence of tertiary amines with the incorporation of acetic acid (AcOH) (Scheme 1). The addition of AcOH lowered the reactivity of tertiary amine through partial protonation, which activated the NCA monomer for rate acceleration with negligible AMM initiation. While the simultaneous addition of AcOH/TEA led to acceptable MW control, in-depth study suggested the coexistence of both

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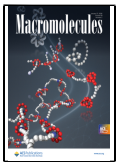

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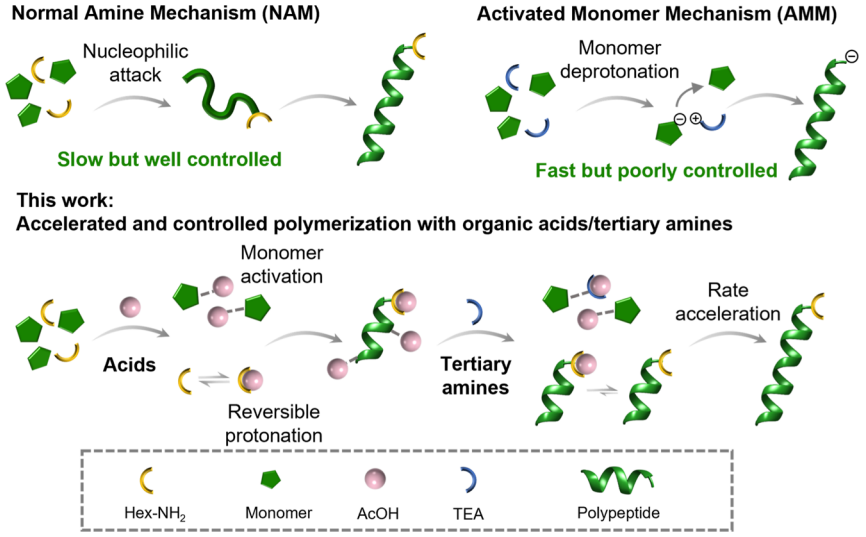
Scheme 1. Illustration of Conventional NCA Polymerization with NAM and AMM and the Polymerization in This Work in the Presence of Organic Acids and Tertiary Amines



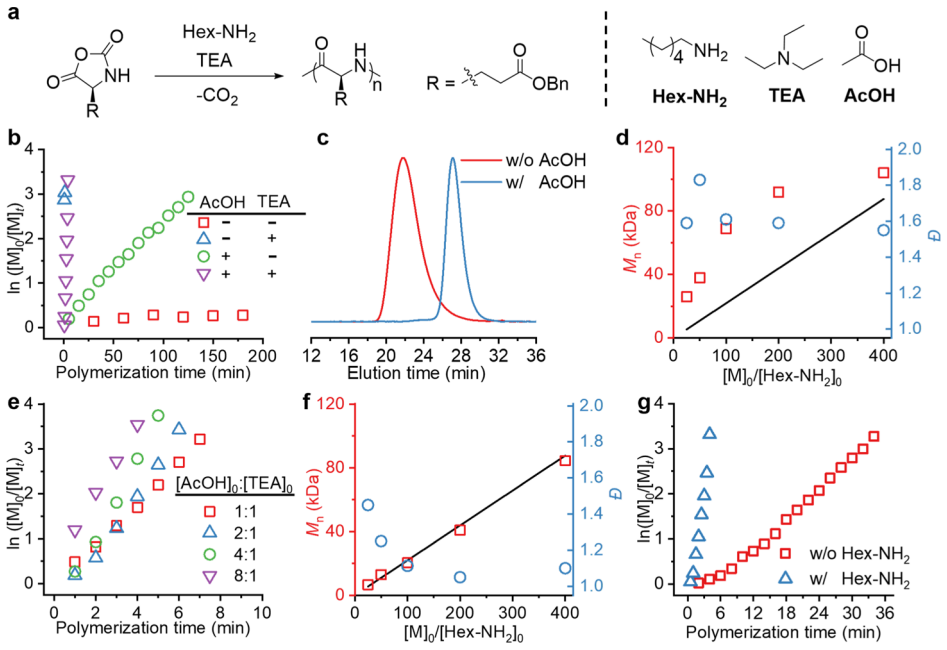


Figure 1. Impact of organic acids on the polymerization of NCA in the presence of Hex-NH_2 and TEA in DCM. (a) Polymerization scheme showing the polymerization of BLG-NCA in the presence of Hex-NH_2 and TEA with or without AcOH. (b) Semilogarithmic kinetic plots of polymerization of BLG-NCA in the presence of Hex-NH_2 with or without the addition of TEA or AcOH. $[M]_0/[\text{Hex-NH}_2]_0$ = 100. (c) Normalized GPC-LS traces of obtained PBLG from the polymerization in the presence of Hex-NH_2 and TEA with or without the addition of AcOH. $[M]_0/[\text{Hex-NH}_2]_0$ = 100. (d) The obtained MWs and dispersity of resulting PBLG from the polymerization at different $[M]_0/[\text{Hex-NH}_2]_0$ ratios in the presence of Hex-NH_2 and TEA. (e) Semilogarithmic kinetic plots of polymerization of BLG-NCA in the presence of Hex-NH_2, TEA, and AcOH at different $[\text{AcOH}]_0/[\text{TEA}]_0$ ratios. $[M]_0/[\text{Hex-NH}_2]_0$ = 100. (f) The obtained MWs and dispersity of resulting PBLG from the polymerization at different $[M]_0/[\text{Hex-NH}_2]_0$ ratios in the presence of Hex-NH_2, TEA, and AcOH. (g) Semilogarithmic kinetic plots of polymerization of BLG-NCA in the presence of TEA and AcOH and TEA with or without the addition of Hex-NH_2. For all experiments, $[M]_0$ = 0.1 M, $[M]_0/[\text{AcOH}]_0/[\text{TEA}]_0$ = 100:100:25.

NAM and AMM initiation. The sequential addition of AcOH/TEA was thus used to suppress AMM initiation, leading to the synthesis of well-defined polypeptides within 10 min. We believe this work not only improves our understanding on the manipulation of NCA polymerization pathways but also offers simple and effective polymerization systems for the efficient preparation of polypeptide materials.

RESULTS AND DISCUSSION

Organic Acid-Mediated Controlled Polymerization in the Presence of Tertiary Amines

To check the impact of organic bases on the polymerization behavior of NCA, a conventional AMM initiator, TEA, was added into the dichloromethane (DCM) solution of γ-benzyl-L-glutamate NCA (BLG-NCA) in the presence of a NAM initiator, *n*-hexylamine (Hex-NH_2) ($[M]_0$ = 0.1 M, $[M]_0$/

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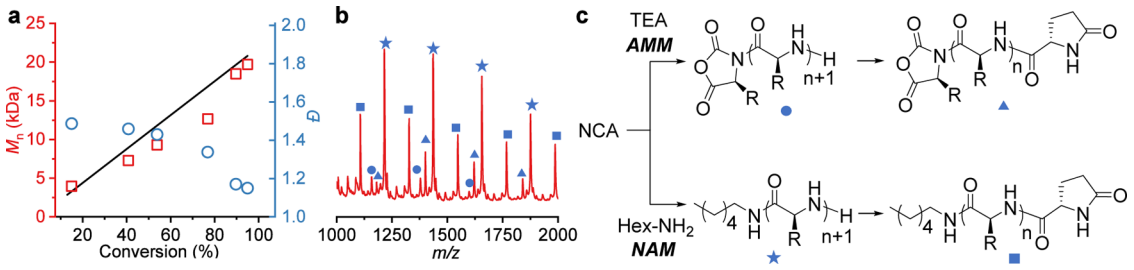

Figure 2. Poor control over MWs for polymerization in the presence of Hex-NH_2, TEA, and AcOH with simultaneous addition. (a) The obtained MWs and dispersity of resulting PBLG from the polymerization in the presence of Hex-NH_2, TEA, and AcOH at various monomer conversions. $[M]_0/[\text{Hex-NH}_2]_0$ = 100. (b) MALDI-TOF spectrum of resulting PBLG obtained from polymerization in the presence of Hex-NH_2, TEA, and AcOH. $[M]_0/[\text{Hex-NH}_2]_0$ = 10. (c) Scheme illustrating the generation of various oligomeric species in b. For both experiments, $[M]_0$ = 0.1 M, $[M]_0/[\text{AcOH}]_0/[\text{TEA}]_0$ = 100:100:25.

$[\text{Hex-NH}_2]_0/[\text{TEA}]_0$ = 100:1:25) (Figure 1a). At low $[M]_0$, the polymerization of BLG-NCA in the absence of AcOH and TEA proceeded in a relatively slow manner even in the low-polarity, chlorinated solvents, reaching only 20% monomer conversion after 40 min that agreed well with literature results (Figure 1b).[36] In contrast, the addition of TEA significantly accelerated the polymerization with NCA conversion >95% after 1 min (Figure 1b). Further tuning of the $[\text{TEA}]_0/[\text{Hex-NH}_2]_0$ ratio revealed a faster polymerization with a higher amount of TEA (Figure S1). While the addition of equimolar Hex-NH_2 and TEA required ∼40 min to complete the polymerization, increasing $[\text{TEA}]_0/[\text{Hex-NH}_2]_0$ to 5 shortened the polymerization to sub-10 min. The polymerization time to reach >95% conversion was 7 and 0.5 min at $[\text{TEA}]_0/[\text{Hex-NH}_2]_0$ ratios of 10 and 50, respectively (Figure S1), confirming the accelerating role of TEA in NCA polymerization.

Despite the significant improvement in polymerization rate, the resulting poly(γ-benzyl-L-glutamate) (PBLG) exhibited broad dispersity ($Đ = M_w/M_n$ = 1.61), which indicated poor polymerization control (Figure 1c). In fact, while the incorporation of TEA reduced the polymerization time at various $[M]_0/[\text{Hex-NH}_2]_0$ ratios to sub-10 min, the obtained MWs of resulting PBLG were 1.2−4.7 times larger than the theoretical values calculated from $[M]_0/[\text{Hex-NH}_2]_0$, with the dispersity varying from 1.55 to 1.83 (Figure 1d, Figure S2, and Table S1). The addition of strong organic bases like TEA inevitably deprotonates NCA monomers, resulting in the uncontrolled polymerization through the AMM pathway. It is therefore necessary to suppress the NCA deprotonation process for the controlled synthesis of polypeptide materials in the presence of tertiary amines.

In order to circumvent NCA deprotonation and AMM polymerization, the addition of protic organic acids is a natural choice. Several research groups reported the catalyzed ROP in the presence of organic acids to prepare polypeptide and polypeptoid materials.[29,37−40] Interestingly, the addition of AcOH at an equimolar ratio of NCA monomers did not significantly slow down the polymerization, where the polymerization reached >95% conversion within 4 min at $[M]_0/[\text{Hex-NH}_2]_0/[\text{AcOH}]_0/[\text{TEA}]_0$ = 100:1:100:25 (Figure 1b). In comparison, while AcOH exhibited a catalytic effect,[29] the polymerization proceeded in a much slower manner in the absence of TEA (Figure 1b). Further increase in $[\text{AcOH}]_0$ slightly slowed down the polymerization rate, but still with >95% monomer conversion within 10 min (Figure 1e). According to previous literature results, the relatively weak acidity of AcOH likely contributed to the rate acceleration rather than inhibition.[29] Therefore, even though the amount of AcOH was larger than that of basic species (i.e., Hex-NH_2 and TEA), the polymerization still proceeded in an accelerated manner. While the addition of AcOH did not significantly alter the polymerization time, its impact on MW control was obvious. The obtained MWs of PBLG at $[M]_0/[\text{Hex-NH}_2]_0$ = 100 was 20.4 kDa (Figure 1c), agreeing well with the theoretical value from $[M]_0/[\text{Hex-NH}_2]_0$ (i.e., 21.9 kDa). Meanwhile, the MWs of polypeptides at various $[M]_0/[\text{Hex-NH}_2]_0$ (25−400) scaled linearly with the predicted values (Figure 1f). All polypeptides exhibited narrow dispersity ($Đ$ < 1.15) except for that at low $[M]_0/[I]_0$ (i.e., 50 and 25), with the GPC trace of the resulting polypeptide at $[M]_0/[I]_0$ = 25 exhibiting an obvious low-MW shoulder peak (Figure S2 and Table S2). Meanwhile, the kinetic profile in the presence of AcOH and TEA resembled that of AcOH-catalyzed polymerization (Figure 1b).[29] The hydrogen-bonding interactions between AcOH and polypeptide chains likely minimized the differences between α-helical and random-coiled chains, contributing to the one-stage kinetics. Finally, it has to be noted that Hex-NH_2 mainly served as initiators even in the presence of TEA and AcOH, as the absence of Hex-NH_2 significantly elongated the polymerization time (Figure 1g), presumably due to the absence of an effective initiation step. While the introduction of AcOH improved the MW control of TEA-initiated polymerization (Figure S3 and Table S3), the bimodal GPC peak suggested the important role of Hex-NH_2 in mediating the controlled polymerization process.

Competing NAM/AMM Initiation in the Presence of Organic Acid/Tertiary Amine

Encouraged by the controlled and accelerated polymerization of NCA in the presence of TEA and AcOH, we further checked the livingness of the TEA/AcOH-catalyzed polymerization by measuring the MWs at various monomer conversions. While the M_n-conversion plot exhibited a linear relationship (Figure 2a), the dispersity of the resulting polypeptides was relatively broad at conversions <80%, ranging from 1.3 to 1.5, which suggested the existence of multiple initiation mechanisms. Indeed, GPC characterization suggested a bimodal distribution of PBLG at early stages of polymerization (i.e., conversion <20%) (Figure S4). Moreover, consistent with the GPC result at $[M]_0/[\text{Hex-NH}_2]_0$ = 25 (Figure S2), a bimodal GPC peak with an obvious low-MW shoulder peak was observed on the GPC trace of the polypeptides obtained in the presence of TEA and AcOH at $[M]_0/[\text{Hex-NH}_2]_0$ = 10 (Figure S4). These results collectively suggested multiple reaction pathways at the early stage of

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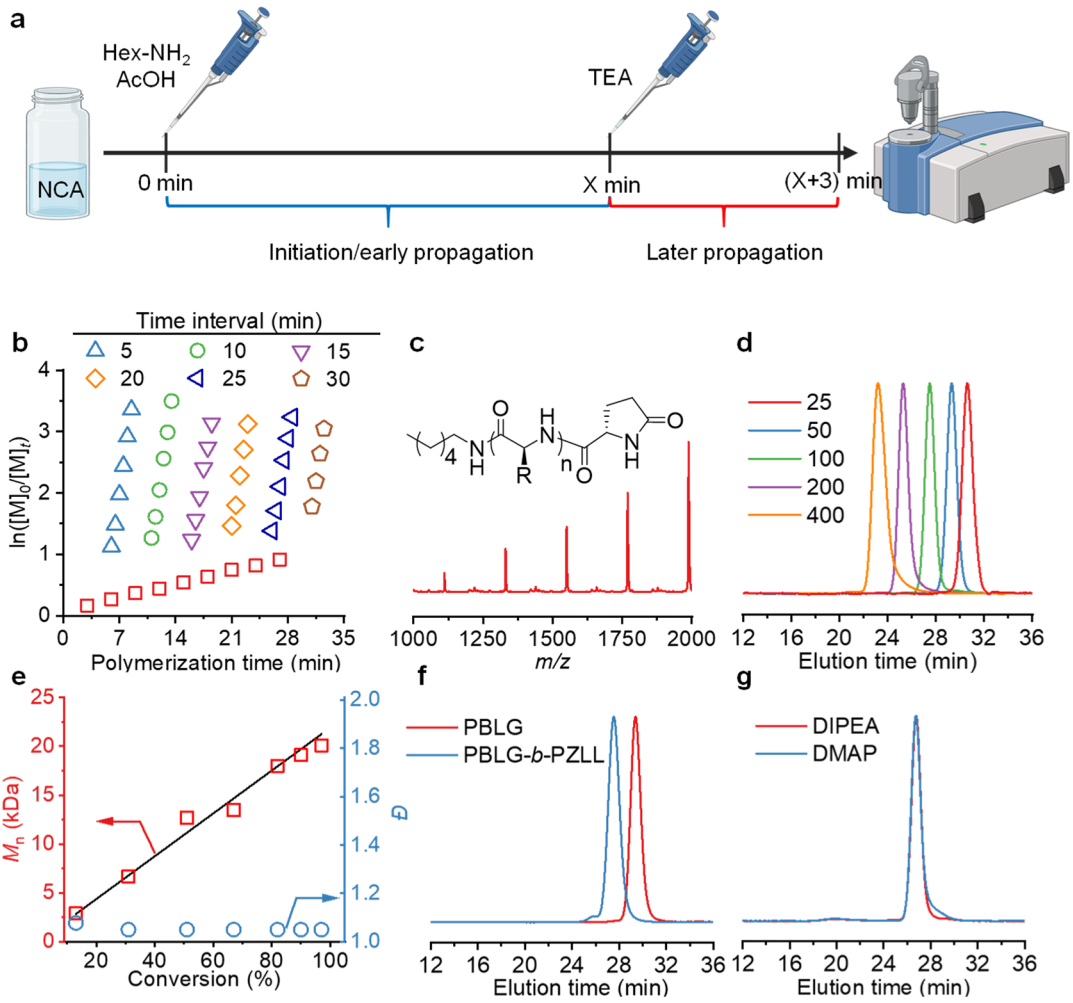

Figure 3. Accelerated and living polymerization initiated by Hex-NH_2 with sequential addition of acids and tertiary amines in DCM. (a) Scheme illustration showing the accelerated and controlled polymerization with sequential addition of AcOH and TEA. (b) Semilogarithmic kinetic plots of polymerization of BLG-NCA in the presence of AcOH with the addition of TEA at various time points. $[M]_0/[Hex\text{-}NH_2]_0$ = 100. The red diamonds represent the polymerization kinetics without the addition of TEA. (c) MALDI-TOF spectrum of resulting PBLG obtained from polymerization with sequential addition of AcOH and TEA. $[M]_0/[Hex\text{-}NH_2]_0$ = 10. (d) Normalized GPC-LS traces of the obtained PBLG from polymerization at various $[M]_0/[Hex\text{-}NH_2]_0$ ratios with sequential addition of AcOH and TEA. (e) The obtained MWs and dispersity of resulting PBLG from the polymerization at various monomer conversions with sequential addition of AcOH and TEA. $[M]_0/[Hex\text{-}NH_2]_0$ = 100. (f) Normalized GPC-LS traces showing the synthesis of diblock copolypeptides PBLG-*b*-PZLL with sequential addition of AcOH and TEA. $[M]_0/[Hex\text{-}NH_2]_0$ = 50 for each block. (g) Normalized GPC-LS traces of the resulting PBLG obtained from the polymerization with sequential addition of AcOH and DIPEA or DMAP. $[M]_0/[Hex\text{-}NH_2]_0$ = 100. For all experiments, $[M]_0$ = 0.1 M, $[M]_0/[AcOH]_0/[TEA]_0$ = 100:100:25. For experiments c-g, the time interval for sequential addition was 5 min.

polymerization, presumably due to the initiation from both Hex-NH_2 and TEA.

To further confirm the multiple reaction pathways, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum of the resulting polypeptide was collected, which revealed oligomeric species from both NAM initiation (i.e., with an *n*-hexyl tail at the C terminus) and AMM initiation (i.e., with a five-membered NCA ring at the C terminus) (Figure 2b). Even in the presence of AcOH, the competing initiation from Hex-NH_2 and the NCA anion led to the chain propagation with two different mechanisms, which deviated from ideal living polymerization behavior (Figure 2c). Only at sufficiently high $[M]_0/[Hex\text{-}NH_2]_0$ or monomer conversions did the two polymeric species reach comparable growth, resulting in the monomodal GPC peak. Therefore, although the addition of TEA/AcOH effectively accelerated the polymerization to generate polypeptides with predictable MWs, complete suppression of the AMM pathway remains challenging, especially at low $[M]_0/[Hex\text{-}NH_2]_0$. The existence of two different C termini also raised issues regarding the functionalization of the polypeptide chain-end or the preparation of block copolymers.

Fast and Controlled Polymerization with Sequential Addition of Acids and Tertiary Amines

To solve the competing AMM initiation issue while taking advantage of the accelerating role of TEA, we sought to temporally decouple the initiation/early propagation stage from the later propagation stage by manually adding Hex-NH_2/AcOH and TEA in a sequential manner (Figure 3a). The first addition of Hex-NH_2/AcOH ($[M]_0/[AcOH]_0$ = 1) not only guaranteed the sole initiation with a NAM but also skipped the two-stage kinetics that is commonly observed for the polymerization in DCM.[16,18] On the other hand, the later incorporation of TEA accelerated the polymerization with

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Table 1. Characterization of Resulting Polypeptides from Polymerization with Sequential Addition of Acids and Tertiary Amines[a]

entry	$[M]_0/[I]_0$	initiator	monomer	tertiary amine	t (min)[b]	$M_{n,\,GPC}$ (kDa)[c]	$M_{n,\,theo.}$ (kDa)	$Đ$[c]
1	25	Hex-NH_2	BLG-NCA	TEA	6	5.3	5.5	1.06
2	50	Hex-NH_2	BLG-NCA	TEA	6	11.6	11.0	1.05
3	100	Hex-NH_2	BLG-NCA	TEA	7	23.7	21.9	1.05
4	200	Hex-NH_2	BLG-NCA	TEA	8	45.3	43.8	1.05
5	400	Hex-NH_2	BLG-NCA	TEA	8	85.2	87.6	1.05
6	100	Hex-NH_2	ZLL-NCA	TEA	8	34.7	26.2	1.07
7	100	Hex-NH_2	POB-NCA	TEA	8	31.8	26.8	1.05
8	100	benzylamine	BLG-NCA	TEA	8	20.2	87.6	1.05
9	100	propargylamine	BLG-NCA	TEA	8	18.8	21.9	1.05
10[d]	50 + 50	Hex-NH_2	BLG-NCA/ZLL-NCA	TEA	8 + 11	10.4/19.7	10.8/24.1	1.05/1.06
11	100	Hex-NH_2	BLG-NCA	DIPEA	8	30.1	21.9	1.05
12	100	Hex-NH_2	BLG-NCA	DMAP	8	27.1	21.9	1.06

[a]All polymerizations were conducted at room temperature in DCM with sequential addition of AcOH and tertiary amines (time interval: 5 min). $[M]_0/[AcOH]_0/[\text{tertiary amines}]_0$ = 100:100:25, $[M]_0$ = 0.1 M. [b]Polymerization time reaching 95% monomer conversion. [c]Determined by GPC; dn/dc = 0.1−0.123. [d]Preparation of diblock copolypeptides through sequential addition of NCA monomers.

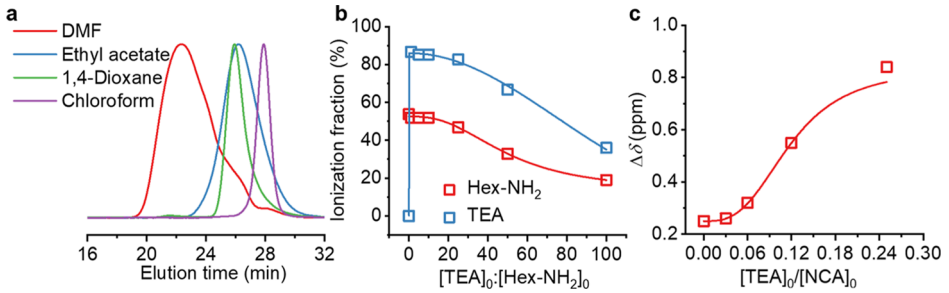

Figure 4. Exploration of polymerization mechanisms in the presence of acids and tertiary amines. (a) Normalized GPC-LS traces of resulting PBLG in various solvents initiated by Hex-NH_2 with sequential addition of AcOH and TEA. (b) The ionization fraction of Hex-NH_2 and TEA in the presence of AcOH at different $[TEA]_0/[\text{Hex-}NH_2]_0$. $[AcOH]_0/[\text{Hex-}NH_2]_0$ = 100. (c) The change in chemical shift of ring N−H protons of NCA at various $[TEA]_0/[NCA]_0$ in the presence of AcOH. $[NCA]_0$ = $[AcOH]_0$ = 0.1 M.

minimal AMM initiation. As expected, the introduction of TEA into the polymerization mixture of NCA/Hex-NH_2/AcOH significantly boosted the polymerization rate regardless of the time intervals between two additions ($[M]_0/[TEA]_0$ = 4) (Figure 3b). In all cases, the conversion of NCA reached 95% within 3 min after the introduction of TEA. GPC analysis showed that the resulting polypeptides possessed predictable MWs along with narrow dispersity ($Đ$ = 1.05) (Figure S5 and Table S4). Furthermore, MALDI-TOF analysis revealed that the C terminus of the resulting PBLG was capped with an *n*-hexyl group, with no polymeric species corresponding to AMM initiation (Figures 3c and S6), confirming that the sequential addition is key to suppressing undesired AMM pathway to achieve rapid and controlled polymerization. Therefore, $[I]_0$ was assigned as the initial concentration of Hex-NH_2 for the later presentation of our studies.

Unlike the poor MW control of polymerization with simultaneous addition of acid/base, the polymerization with sequential addition of AcOH and TEA at varying $[M]_0/[I]_0$ ratios yielded well-defined polypeptides with narrow dispersity ($Đ$ < 1.1), whose M_n values measured by GPC closely matched theoretical predictions (Figure 3d and Table 1). Additionally, the M_n-conversion plot exhibited a linear relationship, suggesting a living polymerization process (Figure 3e). Even at early stages with low conversion, the sequential addition strategy outperformed the simultaneous addition strategy with low dispersity ($Đ$ < 1.1), which was attributed to the minimized AMM initiation. The earlier addition of AcOH likely contributed to the excellent MW control by eliminating the differences between the α-helical and random-coiled chains,[29] even though the conformational transition was obvious from circular dichroism characterization (Figure S7). Since the TEA-initiated polymerization took ∼30 min to finish in the presence of AcOH, the accelerated polymerization with Hex-NH_2/TEA likely outpaced the growth of AMM-initiated polymers, especially when the TEA was added in a later stage. Meanwhile, the change in the acid/base ratio (i.e., $[AcOH]_0/[TEA]_0$ = 1−16) while fixing $[M]_0/[AcOH]_0$ = 1 did not significantly alter the polymerization kinetics or the MW control (Figure S8).

The sequential addition of acid/base was also applied to the polymerization of other NCA monomers, including N^{ε}-benzyloxycarbonyl-L-lysine NCA (ZLL-NCA) and γ-(4-propargyloxy)benzyl-L-glutamate NCA (POB-NCA). Both monomers underwent rapid polymerization to yield well-defined polypeptides (Figure S9 and Table 1). With the suppression of AMM initiation, various primary amines, including benzylamine, propargylamine, and methoxy poly(ethylene glycol) (PEG) amine (5.0 kDa), were successfully employed as NAM initiators to introduce functional handles at the C-terminus (Figure S9 and Table 1). Moreover, sequential addition of monomers enabled the accelerated synthesis of diblock copolymers (PBLG-*b*-PZLL) within 20 min, further confirming the living character of the polymerization (Figure 3f and Table 1). Additionally, the sequential addition was extended to other tertiary amines, which served as kinetic

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modulators with suppressed AMM initiation. The use of *N*,*N*-diisopropylethylamine (DIPEA), whose basicity is comparable with that of TEA (the pK_a of conjugated acid, pK_{aH} = 11.0 and 10.8 for DIPEA and TEA, respectively), exhibited similar kinetic regulation (Figure S10). On the other hand, the addition of a weaker base, 4-dimethylaminopyridine (DMAP, pK_{aH} ~ 9.7), led to slightly slower polymerization that took 10 min to reach 95% conversion. Despite the rate differences, all three polymerizations produced well-defined polypeptides with narrow dispersity ($Đ$ < 1.1) (Figure 3g and Table 1).

Mechanism Studies

Encouraged by the versatile strategy of sequential addition of acid/base for the preparation of polypeptide materials within minutes, we further moved on to explore the underlying mechanism of the kinetic regulation and the suppression of AMM initiation. Similar with the acid-assisted polymerization of NCA and other CCP systems,[26,29] the polarity and hydrogen-bonding ability of solvents significantly influence polymerization behaviors. Controlled polymerization was observed only in chlorinated solvents, such as chloroform, with a low dielectric constant and weak hydrogen-bonding capability (Figure 4a). While the use of ethyl acetate and 1,4-dioxane yielded PBLG with a monomodal distribution, the MWs were larger than theoretical values (Table S5). The use of DMF, on the other hand, generated PBLG with a broad GPC trace bearing a shoulder peak in the low-MW region (Figure 4a). The solvent effect suggested the critical role of molecular interactions, especially hydrogen-bonding interactions, between monomers/polymers and acid/base kinetic modulators. The solvation of these reactants in polar or strong hydrogen-bonding solvents thus blocked the interactions, yielding ill-defined polypeptides.

Since TEA retained its ability to accelerate polymerization even in the presence of excessive AcOH (Figure 1b), we reasoned that the introduction of AcOH enabled fine-tuning of TEA function in NCA polymerization, which suppressed its ability to deprotonate NCA monomers while preserving its potential to activate the monomers via hydrogen bonding. Indeed, proton nuclear magnetic resonance (^{1}H NMR) spectra of TEA/AcOH mixtures at varying molar ratios suggested continuous protonation of TEA with a gradual increase in the $[AcOH]_0/[TEA]_0$ ratio, even when AcOH is in large excess. The downfield shift of methylene protons of TEA was obvious when the $[AcOH]_0/[TEA]_0$ ratio was boosted from 4:1 to 8:1 (Figure S11), indicating the protonation of TEA and the reversible formation of TEA-AcOH salts. Quantitative NMR titration analysis suggested ~80% ionization of TEA at $[TEA]_0/[Hex\text{-}NH_2]_0$ = 25 (Figure 4b). Considering the large excess of AcOH and the low efficiency of AMM initiation by TEA (Table S3), the generation of NCA anion was minimized in our studies. The small fraction of unprotonated TEA in the presence of AcOH thus served as relatively weak hydrogen bonding acceptors, which activated the NCA monomers and thereby accelerated the polymerization. As expected, NMR titration analysis suggested an upfield shift of ring N−H protons of NCA with increasing TEA (Figure 4c). Meanwhile, excessive AcOH also partially protonated the primary amino groups at the propagating chain-end. According to previous studies, AcOH catalyzed the NCA polymerization through the activation of monomers.[29] Even though half of the propagating chain-end was protonated, which slowed the polymerization, the net effect was still rate acceleration. Therefore, the kinetic accelerating role of TEA in our system presumably originated from not only its ability to activate the NCA monomers but also the liberation of propagating chain-ends due to its higher basicity. Quantitative NMR titration indicated that ~53% of primary amines were protonated in the presence of excessive AcOH (i.e., 100 equiv), which dropped to 46.8% upon the addition of 25 equiv of TEA and further decreased to 22% in the presence of 100 equiv of TEA (Figure 4b), validating that TEA could partially deprotonate the propagating chain-ends and restore their nucleophilicity for accelerated polymerization. It must be noted that the use of AcOH with weak acidity is crucial in our system for reversible protonation of TEA and propagating chains, as replacing AcOH with stronger acids, such as trifluoroacetic acid (TFA) or hydrochloric acid (HCl), completely suppressed polymerization with no detectable NCA consumption even with the sequential addition of TEA (Figure S12).

CONCLUSION

In this work, we developed a simple yet highly effective strategy to achieve accelerated and controlled polymerization of NCAs in the presence of tertiary amines through minimizing the AMM pathway with organic acids. This sequential addition of acid/base avoided the initiation from tertiary amines such as TEA, thereby contributing to controlled polymerization progress while taking advantage of the rate acceleration mediated by the organic bases. Well-defined polypeptides (degree of polymerization, DP up to 400) with functionalized C terminus, predictable MWs, and low dispersity ($Đ$ < 1.1) were obtained within 10 min. This approach not only improves our understanding on the NAM/AMM regulation during NCA polymerization but also provides a practical route toward the scalable synthesis of polypeptide materials, boosting the downstream applications in materials science, chemical biology, and biomedical engineering.

ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are openly available in the published article and its online Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.macromol.5c02668.

Materials and instruments, experimental procedures, impact of acid/base ratios on polymerization kinetics, polymerization initiated by TEA, CD characterization during polymerization, additional GPC and MALDI-TOF characterization results, and NMR titration (PDF)

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The authors declare no competing financial interest.

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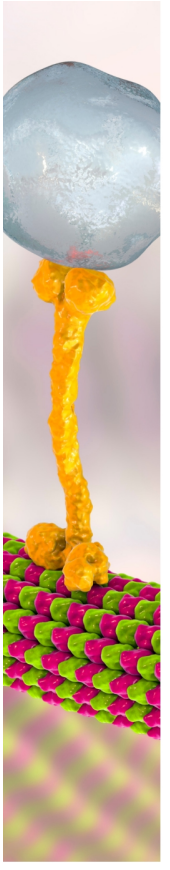

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