CCS Chemistry

RESEARCH ARTICLE

Received: Jan. 22, 2024 | Accepted: Apr. 22, 2024 | Published: Dec. 13, 2024

Accelerated and Controlled Polymerization of *N*-Carboxyanhydrides Assisted by Acids

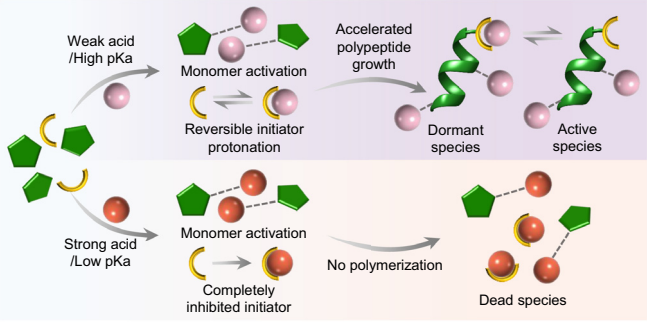
Xingliang Liu[1,2†], Jing Huang[1,2†], Jiaqi Wang[1], Haonan Sheng[1], Zhen Yuan[1], Wanying Wang[3], Wenbin Li[1], Ziyuan Song[3*] & Jianjun Cheng[1,2,4*]

[1]School of Engineering, Westlake University, Hangzhou 310030, [2]Institute of Advanced Technology, Westlake Institute for Advanced Study, Hangzhou, Zhejiang 310024, [3]Institute of Functional Nano & Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Soochow University, Suzhou 215123, [4]Research Center for Industries of the Future, Westlake University, Hangzhou 310030

**Corresponding authors: chengjianjun@westlake.edu.cn; zysong@suda.edu.cn; †*X. Liu and J. Huang contributed equally to this work.

Cite this: *CCS Chem*. **2025**, 7, 2769–2780
DOI: 10.31635/ccschem.024.202403954

The general consensus is that acidic species such as HCl inhibit the polymerization process of *N*-carboxyanhydrides (NCAs), which require their removal to guarantee the successful synthesis of polypeptides. Herein, we show that the impact of organic acids on NCA polymerization was dependent on their pK_a values in dichloromethane (DCM). While strong acids like trifluoroacetic acid (TFA) completely blocks the chain propagation as expected; in contrast, weaker acids such as acetic acid accelerates the polymerization rate. The addition of acids not only protonates the propagating amino groups but also activates NCA monomers, whose balance determined the accelerating or inhibitory effect. Additionally, the acid-assisted polymerization exhibits one-stage kinetics that differs from the conventional cooperative covalent polymerizations, resulting in excellent control over molecular weights even at the accelerating rate. We were able to use this acid-catalyzed mechanism to control the polymerization of nonpurified NCAs. This work shows the possibility of tuning the pH of reaction solution to control NCA polymerization, providing new insights of water-phase polypeptide synthesis.



Keywords: ***N*-carboxyanhydride, polypeptide, organic acid, pK_a value, organocatalysis**

Introduction

Polypeptides from the ring-opening polymerization (ROP) of amino acid *N*-carboxyanhydrides (NCAs) are regarded as the synthetic analog of natural proteins,[1,2] which have shown promising applications in various biomedical areas, including drug delivery, tissue engineering, and antimicrobial applications.[3–12] The advances in living

polymerization of NCA over the last three decades enabled the preparation of polypeptides with predictable molecular weights (MWs) and low dispersity,[13–19] expanding the toolbox to prepare various polypeptide materials. In addition, the recent development of accelerated polymerization strategies not only shortened the polymerization time but also outpaced various side reactions during NCA polymerization.[20–28] Polypeptides can be prepared in a controlled manner even in the presence of aqueous phase,[29–31] which was impossible in conventional polymerization, considering the water-induced NCA degradation.

Despite the exciting advances in NCA polymerization chemistry, the key limitation remains to delicately balance the basic/nucleophilic and acidic/electrophilic species while handling the NCA monomers. While the former induced the degradation of NCA monomers, the latter reacted with the propagating amino groups that blocked the polymerization process.[29,32,33] For instance, the widely used NCA synthetic strategy through the Fuchs-Farthing method,[34,35] involves the phosgenation of corresponding amino acids, generating HCl as one of the major impurities. HCl would inhibit the polymerization of NCAs because its presence protonates amino groups and disfavors the formation of NCA anion, suppressing the polymerization with both normal amine mechanism and activated monomer mechanism.[32,36] Therefore, HCl was commonly used to quench the polymerization in the analysis of polymerization intermediates.[37]

Here, we report that certain organic acids such as acetic acids (AcOH), unlike HCl, accelerated the NCA polymerization in dichloromethane (DCM) rather than inhibiting the process. While acid-catalyzed polymerization has been reported as a strategy to prepare polypeptides and polypeptoids, we found that the NCA polymerization in DCM exhibits a strong dependence on the acidity of the acid, which altered the balance between monomer activation and chain inhibition (Figure 1). AcOH activated NCA monomers through hydrogen bonding (H-bonding) interactions but could not completely block the propagating chains, resulting in an accelerated and living polymerization process. Therefore, polypeptide materials can be prepared from the nonpurified monomers in a fast and controlled manner by shifting the acid-base equilibrium.

Experimental Methods

Polymerization setup and polypeptide characterization

AcOH-accelerated polymerization of NCA was carried out under ambient conditions. For a typical reaction, AcOH (2.17 μL, 0.038 mmol) was mixed with the DCM solution of γ-benzyl-L-glutamate NCA (BLG-NCA) (10 mg, 0.038 mmol) into which the DCM solution of *n*-hexylamine (Hex-NH_2) (0.076 M, 5 μL, 0.38 μmol) was added to start the polymerization ($[M]_0$ = $[AcOH]_0$ = 0.1 M, $[M]_0/[I]_0$ = 100). After >99% conversion of NCA as monitored by Fourier-transform infrared (FTIR) spectrometry, the resulting polymers were purified by precipitation in

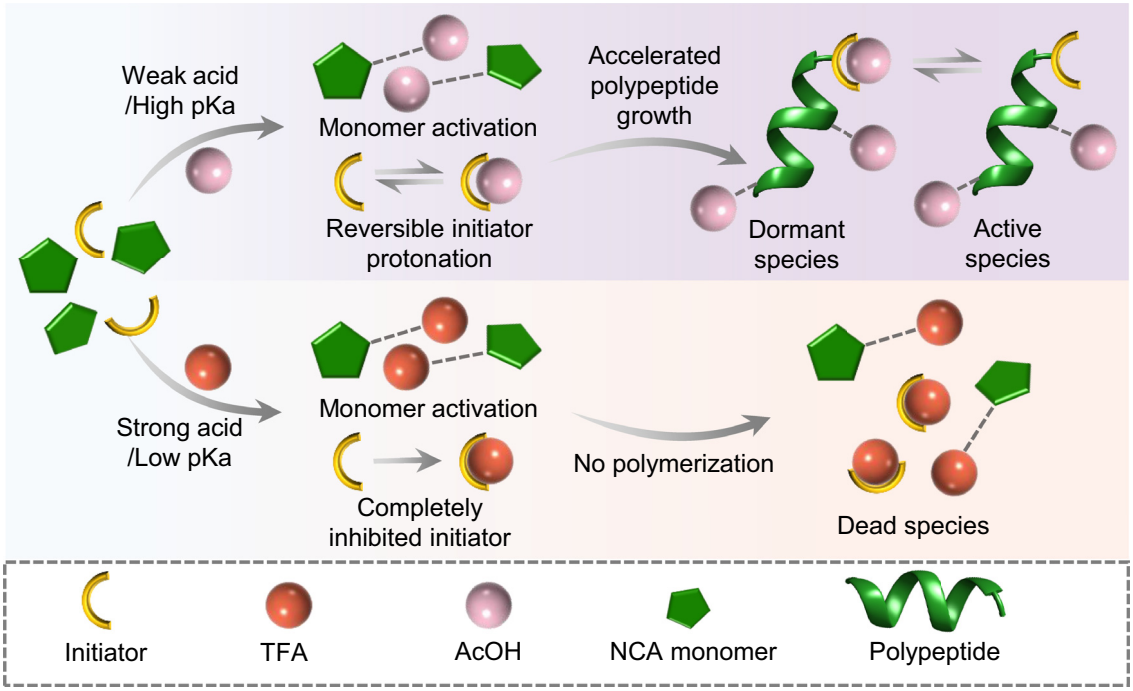


Figure 1 | *Scheme illustrating the pK_a-dependent accelerating/inhibitory effect of organic acids on the polymerization of NCA. NCA,* N-*carboxyanhydride.*

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

hexane/ether (1:1, v/v) and dried under vacuum. The obtained polypeptides were dissolved in *N*,*N*-dimethylformamide (DMF) containing 0.1 M LiBr, filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane (Thermo Fisher Scientific, Waltham, MA, United States), and analyzed by gel permeation chromatography (GPC). Polymerization in other solvents, with other monomers, initiators ($[M]_0/[I]_0$ = 50–200), and acids ($[acid]_0/[M]_0$ = 0.01–2) were conducted in a similar way. In order to check the MWs at different monomer conversions, the polymerization was terminated at different time intervals by the addition of trifluoroacetic acid (TFA; 2.5 vol %). The polypeptides were then collected by precipitation, dried, and dissolved in DMF containing LiBr (0.1 mol/L) for GPC analysis. The secondary structure analysis of polypeptides was conducted similarly, but diluted by 100 times with DCM after quenching. Only the CD spectra at $\lambda > 220$ nm were measured because of the absorbance of DCM at low-wavelength region.

Polymerization of nonpurified BLG-NCA

To a pressure vessel with a heavy wall, BLG (10.0 g, 42.1 mmol), tetrahydrofuran (THF) (150 mL), and methyloxirane (13.0 mL, 169 mmol) were added sequentially while being stirred. Triphosgene (6.3 g, 21.1 mmol) was then added in one portion and the vessel was sealed immediately. The amino acid gradually disappeared in ~30 min with a noticeable heat release. The reaction was subsequently stirred at room temperature for ~1.5 h, then dried under vacuum. The nonpurified NCA could be stored at −20 °C for at least 1 year without significant degradation.

Polymerization of nonpurified NCA with an acid-base equilibrium was carried out under ambient conditions. Typically, HCl (3 μL, 0.038 mmol) was mixed with the DCM solution of nonpurified BLG-NCA (10 mg, 0.038 mmol) into which the DCM solution of Hex-NH_2 (0.076 M, 5 μL, 0.38 μmol) was added. After thorough mixing, NaOAc (3.12 mg, 0.038 mmol) was added to the mixture to convert the inhibitory HCl into catalytic AcOH ($[M]_0 = [HCl]_0 = [NaOAc]_0$ = 0.1 M, $[I]_0$ = 1 mM).

Nuclear magnetic resonance (NMR) titration experiments

NMR titration experiments were conducted to probe the molecular interactions between the reactants during NCA polymerization, including BLG-NCA, Hex-NH_2, AcOH, and chain-end-mimicking α,γ-dibenzyl-L-glutamate (DBLG). To elucidate the NCA/acid interactions, BLG-NCA (10.00 mg, 0.038 mmol) was dissolved in CD_2Cl_2 (380 μL), into which varying amounts of AcOH were added (from 0.02 to 9.28 mg) to adjust the $[AcOH]_0/[NCA]_0$ ratio from 0.01 to 1. The chemical shifts of α-H, the side-chain benzyl protons, and the ring N–H at different $[AcOH]_0/[NCA]_0$ ratios were recorded.

Other NMR titration experiments like Hex-NH_2/acid interactions and DBLG/acid were conducted similarly. For Hex-NH_2/acid experiments, the fraction of Hex-NH_2 protonation was quantified by the chemical shift of α-H of Hex-NH_2, calculated according to the following equation:

$$\delta_m = \delta_b + (\delta_s - \delta_b) \cdot X_s$$

where δ_b and δ_s are the standard chemical shifts of α-H in the amine (–NH_2) and ammonium (–NH_3^+) forms of Hex-NH_2, respectively, and δ_m is the chemical shift of α-H under certain conditions. The mole fraction of protonated amine, X_s, was then calculated from the equation. [38]

Results and Discussion

Accelerated polymerization assisted by AcOH

It has been reported that ammonium salts serve as initiators for the controlled polymerization of NCA or *N*-phenoxycarbonyl amino acids under certain conditions.[14,39,40] While the addition of excessive acids is generally believed to slow down or completely prohibit NCA polymerization,[32,36] organic acids are common catalysts used for polyester synthesis, which activates the monomers through protonation or H-bonding interactions.[41–46] Considering the structural similarity of NCA with lactones, we reasoned that the addition of acids would also activate NCA. Nevertheless, HCl generated during the synthesis of NCA deactivates the nucleophilic initiators or propagates polypeptide chains during NCA polymerization, because the basic nature of the propagating chain-ends would react with the relatively strong acids (i.e., HCl) leading to chain inhibition even with potentially activated monomers. On the other hand, it has been reported that the amine group still has sufficient activity towards *N*-hydroxy succinimide ester for nucleophilic substitution at pH 4-5, when amine is only partially protonated with a considerable fraction staying in its nucleophilic state.[47] Therefore, we hypothesized that a weak organic acid may still serve as an accelerating agent for NCA polymerization, which activates the monomer while still maintaining sufficiently high amine chain-end reactivity.

To verify our hypothesis, AcOH with a high pK_a value (~4.78)[48] was first added into the DCM solution of BLG-NCA and Hex-NH_2 ($[M]_0$ = 0.1 M, $[M]_0/[I]_0/[AcOH]_0$ = 100:1:100) (Figure 2a). FTIR characterization revealed the complete disappearance of NCA anhydride peaks at 1857 and 1790 cm^{-1} after 120 min in the presence of AcOH (Figure 2b). In contrast, the NCA conversion was only 20% in the absence of AcOH after 120 min (Figure 2b), suggesting the role AcOH played for the acceleration of the NCA polymerization. Additionally, NCA was stable in the presence of AcOH without the addition of Hex-NH_2

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

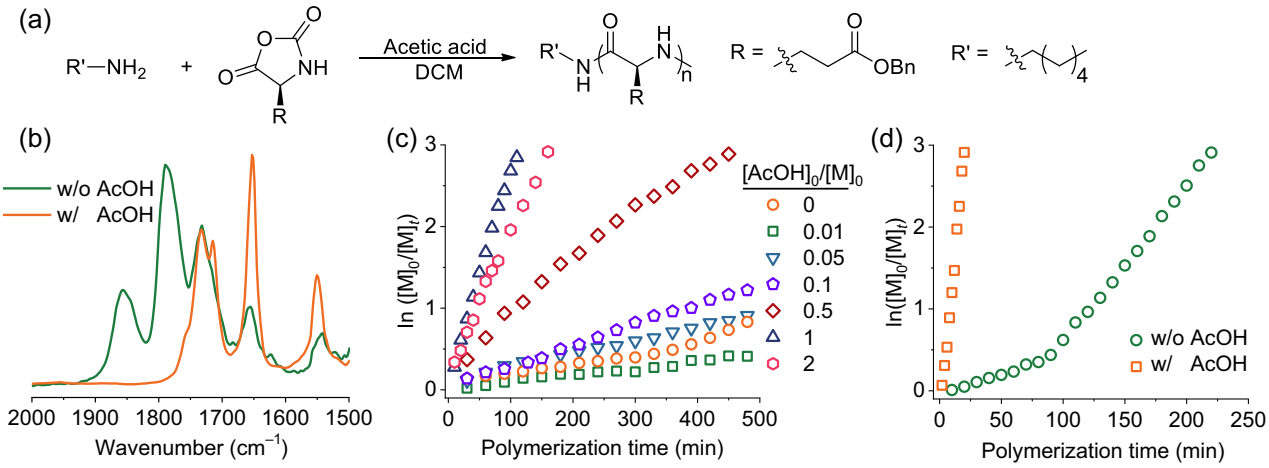


Figure 2 | *AcOH-accelerated polymerization of NCAs. (a) Synthetic route to polypeptides through AcOH-accelerated polymerization of BLG-NCA. (b) Overlaid FTIR spectra showing the polymerization of BLG-NCA initiated by Hex-NH_2 after 120 min in the absence or presence of AcOH in DCM. $[M]_0/[I]_0/[AcOH]_0$ = 100/1/100, $[M]_0$ = 0.1 M. (c) Semilogarithmic kinetic plot of polymerization of BLG-NCA in DCM initiated by Hex-NH_2 at various $[AcOH]_0$. $[M]_0/[I]_0$ = 100, $[M]_0$ = 0.1 M. (d) Semilogarithmic kinetic plot of polymerization of BLG-NCA in DCM initiated by Hex-NH_2 at low $[M]_0/[I]_0$ in the absence or presence of AcOH. $[M]_0/[I]_0/[AcOH]_0$ = 50/1/100, $[M]_0$ = 0.1 M. AcOH, acetic acid; NCAs, N-carboxyanhydrides; BLG-NCA, γ-benzyl-L-glutamate-NCA; Hex-NH_2, n-hexylamine; DCM, dichloromethane; FTIR, Fourier-transform infrared*

initiator (Supporting Information Figure S1), ruling out the possibility that AcOH served as an accelerated initiator.

The acceleration effect of AcOH was dependent on the amount of the acid added, which was revealed by the kinetics analysis of BLG-NCA polymerization and monitored in situ by FTIR. This assay showed a low rate with 47% BLG-NCA conversion after 7 h ($[M]_0$ = 0.1 M, $[M]_0/[I]_0$ = 100:1) in the absence of AcOH (Figure 2c). The addition of a small amount of AcOH, with an equimolar concentration to the initiator, slightly slowed down the polymerization in which only 30% of NCA was consumed after 7 h. The increase in the $[AcOH]_0/[M]_0$ ratio accelerated the NCA polymerization, with the NCA conversion of 56%, 67%, and 94% after 7-h polymerization at $[AcOH]_0/[M]_0$ = 0.05, 0.1, and 0.5, respectively. The polymerization reached the highest rate (maximum) at $[AcOH]_0/[M]_0$ = 1, with an equimolar concentration of AcOH and monomer, where the polymerization reached >95% conversion within 2 h (Figure 2c and Supporting Information Figure S2). A further increase in the $[AcOH]_0/[M]_0$ ratio to 2, however, slightly slowed down the polymerization kinetics. Additionally, with the same batch of BLG-NCA, AcOH exhibited similar accelerating behavior with crown ether (CE), 18-crown 6-ether, which led to faster kinetics in comparison with conventional catalytic polymerization systems in DCM, including tetrabutylammonium acetate (TBAA) initiating system and *N,N′*-bis[3,5-bis(trifluoromethyl)phenyl]thiourea catalytic system (TU-S) (Supporting Information Figure S3). Notably, the fastest kinetics was achieved at $[AcOH]_0/[M]_0$ = 0.1 in *N*-thiocarboxyanhydrides (NTAs)/amino acid *N*-substituted NCAs (NNCAs) polymerization in nonpolar solvents and achieved at $[AcOH]_0/[M]_0$ = 2 in *N*-substituted glycine NTAs (NNTAs) polymerization in polar solvent.[49–54] The latter required a much higher dosage of organic acids owing to their consumption as polar solvents. The difference in kinetics among various monomers indicated different polymerization mechanisms.

The optimal ratio of AcOH was also dependent on the $[M]_0/[I]_0$ since similar kinetics was observed at $[AcOH]_0/[M]_0$ = 1 and 2 for a 50-mer polypeptide synthesis (Supporting Information Figure S2). The kinetic studies of AcOH-mediated polymerization also presented a linear correlation with a slope of 0.05, indicating a first-order kinetic dependence of the polymerization rate dependence on initiator concentration, consistent with features of living polymerization (Supporting Information Figure S4). In addition to $[AcOH]_0$, $[M]_0$ played an important role in the polymerization kinetics, consistent with the previous studies of polymerization in DCM.[55] The increase in $[M]_0$ significantly accelerated the polymerization, as the polymerization rapidly completed within 25 min in the presence of AcOH at $[M]_0$ = 0.2, 0.3, and 0.4 M (Supporting Information Figure S5).

In contrast to the previous reports on the cooperative covalent polymerization (CCP) in DCM,[22,55–58] the kinetic plot of AcOH-accelerated polymerization did not show an obvious two-stage kinetics. In order to further study the detailed kinetic profiles, the polymerization of BLG-NCA was conducted at low $[M]_0/[I]_0$ to clearly reveal the

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

early-stage kinetics. As shown in Figure 2d, the polymerization in the absence of AcOH exhibited a clear two-stage kinetics, agreeing well with previous studies.[22] The two-stage kinetics of CCP originated from the change in secondary structure, where the folding of polypeptides into α-helices accelerated the polymerization that outpaced the random-coiled chains.[22] In sharp contrast, the polymerization exhibited one-stage kinetics in the presence of AcOH (Figure 2d), which was further confirmed by the in situ NMR kinetic plot (Supporting Information Figure S6). Interestingly, CD characterization suggested the formation of a random-coiled structure during AcOH-accelerated polymerization (Supporting Information Figure S7), excluding the possibility that there was no conformational change in the presence of AcOH. Therefore, the addition of AcOH altered the polymerization profile by accelerating both the random-coiled and α-helical propagating chains, resulting in a relatively stable rate constant throughout the polymerization process.

Improved control over MWs originated from the one-stage kinetics

One of the main limitations of CCP is the bimodal distribution of resulting polypeptides, which originates from the two-stage kinetics with a faster secondary stage.[55,58] The propagating chains entering the second stage outgrow those staying in the first stage, resulting in an obvious shoulder peak at the low-MW side on the GPC trace, especially in some fast CCP systems at low $[M]_0/[I]_0$.[22,59] The design of even faster CCP systems was thus, disfavored due to the concerns in MW control. Previous literature had relied on the use of α-helical macroinitiators or cosolvents to skip the first stage or ameliorate the two-stage kinetic profile, respectively.[29,59,60]

The AcOH-accelerated polymerization with accelerated, one-stage kinetics offers a promising strategy to solve the rate/MW-control dilemma, as all propagating chains grew simultaneously at a fast rate in the presence of AcOH. The plot of MWs against monomer conversion revealed a linear relationship, suggesting a living polymerization process (Figure 3a). Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry indicated negligible degradation of propagating amino groups (Supporting Information Figure S8), which was attributed to the accelerated rate. Notably, though carboxylate-initiated ROP of NCAs has been previously reported,[28] no carboxylate end groups were observed in our study, suggesting that the polypeptides were exclusively initiated by the Hex-NH_2 initiator even in the presence of a high concentration of AcOH. The uniform initiation was attributed to the higher nucleophilicity of amino groups compared to the carboxylate groups. The obtained MWs of resulting polypeptides agreed well with the theoretical values when the feeding $[M]_0/[I]_0 \leq 200$, with a narrow dispersity observed for all polymerizations ($Đ = M_w/M_n < 1.10$) (Figure 3b and Table 1). Additionally, using commercially available TBAA and CE results in a significant deviation from the targeted MWs compared to AcOH (Supporting Information Figure S9 and Table S1). At even higher $[M]_0/[I]_0$, the polymerization slowed down significantly such that the MWs obtained were lower than the theoretical values. An increase in $[M]_0$ was, therefore, essential to warranty the fast kinetics to produce poly(γ-benzyl-L-glutamate) (PBLG) 400-mer (Supporting Information Figure S10). Notably, the molecular weight distribution (MWD) of

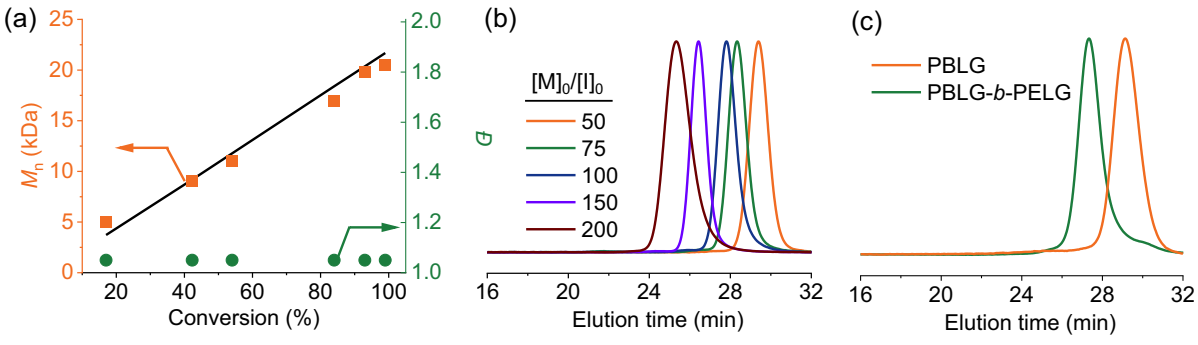


Figure 3 | *Molecular weight control of AcOH-accelerated polymerization. (a) The obtained MWs and dispersity at various monomer conversions during AcOH-accelerated polymerization of BLG-NCA initiated by Hex-NH_2 in DCM. $[M]_0/[I]_0/[AcOH]_0$ = 100/1/100, $[M]_0$ = 0.1 M. (b) Normalized GPC-LS traces of the obtained PBLG from AcOH-accelerated polymerization at different $[M]_0/[I]_0$ ratios. (c) Normalized GPC-LS traces showing the synthesis of diblock copolypeptides PBLG-b-PELG in the presence of AcOH. $[M]_0/[I]_0/[AcOH]_0$ = 50/1/100, $[M]_0$ = 0.1 M. AcOH, acetic acid; MWs, molecular weights; BLG-NCA, γ-benzyl-L-glutamate-* N*-carboxyanhydride; Hex-NH_2, n-hexylamine; DCM, dichloromethane; GPC-LS, gel permeation chromatography-light scattering; PBLG-b-PELG, poly(γ-benzyl-L-glutamate)-b- poly(ethylenediamine-L-glutamate).*

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

Table 1 | *Characterization of the Resulting PBLG from the Polymerization of BLG-NCA at Various $[M]_0/[I]_0$ in the Presence of AcOH*[a]

Entry	$[M]_0/[I]_0/[AcOH]_0$	t (min)[b]	$M_{n,GPC}$ (kDa)[c]	$M_{n,theo.}$ (kDa)	$Đ$[c]
1	100/1/0	1440	19.9	21.9	1.12
2	50/1/100	60	11.1	10.9	1.05
3	75/1/100	70	16.5	16.4	1.05
4	100/1/100	120	20.5	21.9	1.05
5	150/1/100	300	32.4	32.8	1.05
6	200/1/100	480	41.2	43.8	1.05

[a] All polymerizations were conducted at room temperature in DCM with BLG-NCA as the monomer and Hex-NH_2 as the initiator. $[M]_0$ = 0.1 M.
[b] Polymerization time reaching 95% monomer conversion.
[c] Determined by GPC; dn/dc = 0.104.

PBLG, poly(γ-benzyl-L-glutamate); BLG-NCA, γ-benzyl-L-glutamate-*N*-carboxyanhydride; AcOH, acetic acid; DCM, dichloromethane; Hex-NH_2, n-hexylamine; GPC, gel permeation chromatography.

polypeptides obtained at $[M]_0/[I]_0 < 50$ was significantly improved in the presence of AcOH (Supporting Information Figure S11 and Table S2), substantiating the improved MW control of AcOH-accelerated polymerization. The accelerated, controlled polymerization in the presence of AcOH was further extended to other monomers and initiators. Well-defined polypeptides were obtained from the polymerization of N^{ε}-carboxybenzyl-L-lysine NCA (ZLL-NCA) and γ-(4-propargyloxy)benzyl-L-glutamate NCA (PPOBLG-NCA), with the latter having a functional alkyne side chain, enabling further modifications (Supporting Information Figure S12 and Table S3). Additionally, the livingness of AcOH-accelerated chains allowed for efficient chain extension to prepare block copolypeptides. GPC characterization revealed a clear peak shift with a high blocking efficiency (Figure 3c and Supporting Information Table S4). Other primary amines, including benzylamine, propagylamine, and methoxy poly(ethylene glycol) amine, were also used as the initiator for the AcOH-accelerated polymerization (Supporting Information Figure S12 and Table S3). We found that the successful incorporation of the functional group at the C terminus allowed for further polypeptide functionalization (Supporting Information Figure S13).

pK_a-Dependent acceleration or inhibitory effect of organic acids

The accelerated polymerization of NCA in the presence of AcOH, in contrast to our previous understanding of the inhibitory effect of acid, prompted us to further explore the impact of more organic acids on the polymerization profile (Figure 4a). The addition of TFA, an acid with a much lower pK_a value (pK_a ~ 0.52) than AcOH,[48] completely inhibited the polymerization with negligible monomer conversion after 9 h (Figure 4b), suggesting that the pK_a value of the acid played an important role in determining the polymerization behaviors. With the addition of bromoacetic acid (BrA, pK_a ~ 2.90), formic acid (FA, pK_a ~ 3.77), and benzoic acid (BnA, pK_a ~ 4.20),[48] low conversions of NCA monomers were observed after 9 h, with the monomer conversion increasing monotonously with weaker acidity, reaching ~33%, 56%, and 81%, respectively. Finally, the selection of even weaker acids exhibited negligible differences in the acceleration effect, since the addition of propanoic acid (PrA, pK_a ~ 4.87) and trimethylacetic acid (TMA, pK_a ~ 5.03) accelerated the polymerization in a similar way to that of AcOH,[48] but at a slightly slower rate.

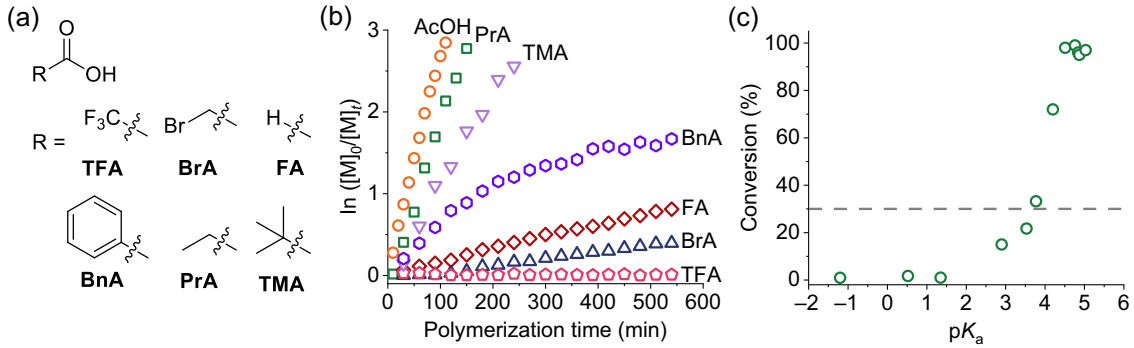


Figure 4 | *pK_a-Dependent acceleration/inhibitory effect of organic acids. (a) Chemical structures of various organic acids with different pK_a values. (b) Semilogarithmic kinetic plot of polymerization of BLG-NCA in DCM initiated by Hex-NH_2 in the presence of various organic acids. $[M]_0/[I]_0/[acid]_0$ = 100/1/100, $[M]_0$ = 0.1 M. (c) The plot of the 4-h NCA conversion against the pK_a value of added organic acids. The grey, dashed line indicates the 4-h NCA conversion in the absence of any organic acids. $[M]_0/[I]_0/[acid]_0$ = 100/1/100, $[M]_0$ = 0.1 M. BLG-NCA, γ-benzyl-L-glutamate-*N*-carboxyanhydride; DCM, dichloromethane; Hex-NH_2, n-hexylamine.*

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

Table 2 | *Characterization of Resulting PBLG Initiated from the Polymerization of BLG-NCA Accelerated by Various Organic Acid*[a]

Entry	Organic Acid[b]	t (min)[c]	$M_{n,GPC}$ (kDa)[d]	$Đ$[d]
1	PrA	160	22.6	1.05
2	TMA	240	21.0	1.05
3	IA	240	22.2	1.05
4	CA	240	24.7	1.06
5	BnA	720	20.1	1.14

[a] All polymerizations were conducted at room temperature in DCM with BLG-NCA as the monomer and Hex-NH_2 as the initiator. The theoretical MW was 21.9 kDa. $[M]_0/[I]_0/[acid]_0$ = 100/1/100, $[M]_0$ = 0.1 M.
[b] PrA, propanoic acid; TMA, trimethylacetic acid; IA, isobutyric acid; CA, cyclohexylacetic acid; BnA, benzoic acid; PBLG, poly(γ-benzyl-L-glutamate); BLG-NCA, γ-benzyl-L-glutamate-*N*-carboxyanhydride; DCM, dichloromethane; Hex-NH_2, n-hexylamine; MW, molecular weight; GPC, gel permeation chromatography.
[c] Polymerization time reaching 95% monomer conversion.
[d] Determined by GPC; d*n*/d*c* = 0.104.

By plotting the monomer conversion after 4-h polymerization against the pK_a of various acids, it was clear that the polymerization kinetics exhibited a strong dependency on the acidity (Figure 4c and Supporting Information Table S5). While stronger acids (pK_a < 1.5) completely inhibited the polymerization, acids with higher pK_a values showed an acceleration effect, starting with pK_a > 4. The conversion of NCA was >95% within 4 h in the presence of acids with pK_a > 4.5. It should be noted that the polymerization kinetics in the presence of FA resembled that in the absence of any organic acids. Therefore, FA was regarded as a model organic acid to differentiate the organic acids with acceleration or inhibitory effects depending on their acidity. GPC analysis revealed well-defined polypeptides with low dispersity ($Đ = M_w/M_n < 1.2$) for all acid-accelerated polymerizations (Table 2).

Mechanism studies

The pK_a-dependent effect of acids on NCA polymerization behaviors encouraged us to elucidate the underlying mechanism. We first checked the solvent effect of AcOH-accelerated polymerization. The accelerated and controlled polymerization of NCA was only observed in solvents with low polarity and weak hydrogen bonding ability such as DCM and chloroform (Figure 5a and Supporting Information Figure S14). While the polymerization in polar DMF in the presence of AcOH exhibited a relatively fast reaction rate (Supporting Information Figure S14), the resulting polypeptides presented a bimodal MWD (Figure 5a and Supporting Information Table S6), likely due to the presence of two polymerization processes initiated both by the amine and ammonium form of Hex-NH_2. The polymerization reaction carried out in THF, on the other hand, proceeded at a much slower rate and generated polypeptides with a broad MWD (Figure 5a and Supporting Information Figure S14 and Table S6). Since there were significant solvations of NCA monomers and propagating polypeptide chains in DMF and THF,[55] the solvent dependence suggested that molecular interactions played important roles during the AcOH-accelerated effect.

The rate-limiting step of NCA polymerization was the nucleophilic attack of propagating amines on the NCA anhydride.[44] To simplify the system, the kinetics of the ring-opening reaction was monitored in the presence and absence of AcOH, with the mixing of BLG-NCA and chain-end-mimicking DBLG at a ratio of 1:10 (Supporting Information Figure S15).[59] The presence of AcOH significantly accelerated the ring-opening reaction, with the NCA being completely consumed within 6 min. In sharp contrast, the conversion of NCA was only 15% after 40 min in the absence of AcOH, suggesting that the acceleration effect mainly involved the local interactions at the *N* terminus, with negligible contribution from the overall polymeric structure. Moreover, the acceleration effect was verified through a model reaction between DBLG and maleic anhydride (Supporting Information Figure S16), indicating that the pK_a-dependence was a general phenomenon regardless of the structure of the electrophile.

Learning from the organo-accelerating ROP to prepare polyesters and polycarbonates,[41,43,45,46] we reason that the acceleration effect of AcOH originated from the carbonyl activation of NCA monomers (Figure 1). The H-bonding interactions between NCA and AcOH lowered the energy of the lowest unoccupied molecular orbital (LUMO) of the anhydride groups,[61] leading to rate enhancement. On the other hand, the pK_a-dependent acceleration/inhibitory effect of acids likely resulted from the protonation of propagating amino groups (Figure 1). While weaker acids such as AcOH reversibly protonated the amino groups at the chain end, stronger acids like TFA blocked the nucleophilic reaction by completely protonating the terminal amines. To verify our hypothesis, ^{1}H NMR and ^{13}C NMR titration experiments were performed to study the interactions between NCA and AcOH.[45,59,62] The addition of AcOH into the solution of BLG-NCA induced an obvious downfield shift of the proton around 6.5 ppm in ^{1}H NMR and C2 carbon around 152 ppm in ^{13}C NMR (Figure 5b and Supporting Information Figures S17 and S18), corresponding to the deshielding of the ring N–H proton.[63] The shift of α-H and side-chain benzyl protons, however, was negligible compared to ring N–H, suggesting that the NCA interacted with AcOH through H-bonding interactions at the ring amide structure. The slight downfield shift of C5 carbon of NCA around 172 ppm showed the

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

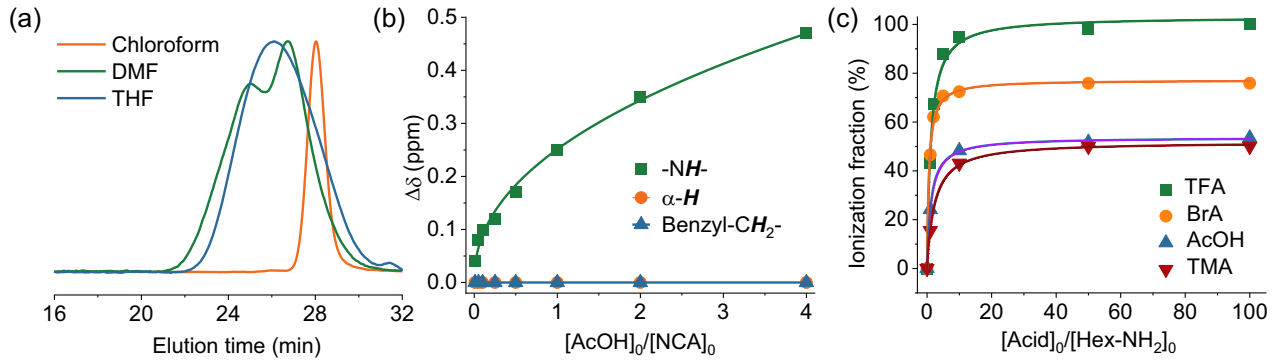


Figure 5 | *Elucidation of accelerating/inhibitory mechanisms. (a) Normalized GPC-LS traces of the obtained PBLG in various solvents initiated by Hex-NH_2 in the presence of AcOH. $[M]_0/[I]_0/[AcOH]_0$ = 100/1/100, $[M]_0$ = 0.1 M. (b) The change in chemical shift of various protons in NCA at various $[AcOH]_0/[NCA]_0$ ratios. $[NCA]_0$ = 0.1 M. (c) The ionization fraction of Hex-NH_2 in the presence of various organic acids at different $[Acid]_0/[Hex\text{-}NH_2]_0$. GPC-LS, gel permeation chromatography-light scattering; PBLG, poly(γ-benzyl-L-glutamate); AcOH, acetic acid; NCA, N-carboxyanhydride; Hex-NH_2, n-hexylamine.*

activation of the C5 carbonyl group via a through-bond conductivity effect (Supporting Information Figure S18).[64,65] The α-carbon was also downfield-shifted due to the through-bond conductivity effect (Supporting Information Figure S18).[64,65] The interactions activated the carbonyl group of the NCA ring, rendering it more susceptible to nucleophilic attack, which facilitated the ring-opening reaction. Moreover, TFA induced an even larger shift of ring N–H (Supporting Information Figure S17), indicating an even stronger affinity with NCA.[66] Nevertheless, the protonation of propagating primary amino groups, as calculated from the chemical shift of methylene groups adjacent to amino groups in Hex-NH_2 (see Supporting Information Figure S19 for details),[38,67] was >90% in the presence of 10 equiv of TFA (Figure 5c). Therefore, even though TFA exhibited a stronger activation of NCA monomer than AcOH, the complete blocking of nucleophilic amines led to the inhibitory effect. In comparison, organic acids with weaker acidity showed partial protonation of Hex-NH_2 even at $[acid]_0/[Hex\text{-}NH_2]_0$ = 100 (Figure 5c). For instance, 47% Hex-NH_2 stayed in the nucleophilic form in the presence of 100 equiv of AcOH, which allowed for accelerated polymerization with activated monomer. Additionally, the multiplicity of the methylene groups indicated complete protonation of Hex-NH_2 in the presence of TFA (Supporting Information Figure S19). The simultaneous growth of all polypeptide chains, as evidenced by the low dispersity of resulting polypeptides, presumably originated from the fast, reversible exchange of protons among all propagating chains.[14] Moreover, a density functional theory (DFT) calculation revealed a significantly lower Gibbs free energy of the transition state of the ring-opening reaction in the presence of AcOH (10.1 kcal/mol) compared to the absence of AcOH (16.3 kcal/mol) (Supporting Information Figure S20), confirming the accelerating role of AcOH. In addition, AcOH promoted the decarboxylation of carbamate species to accelerate the polymerization, as evidenced by lower Gibbs free energy of the transition state (TS2: 34.8 kcal/mol vs TS3-AcOH: 12.4 kcal/mol) (Supporting Information Figure S21).

The one-stage kinetics of AcOH-accelerated polymerization was attributed to H-bonding interactions between acids and polypeptide chains, which minimized the binding differences of NCA with α-helical and random-coiled propagating chains.[58,60] NMR titration studies suggested that various H-bonding interactions occurred between AcOH and DBLG at the backbone and side-chain carbonyl groups (Supporting Information Figure S22), substantiating the use of an equimolar ratio of AcOH to NCA to reach the fastest kinetics (Figure 2c). Without sufficient AcOH in the system, the competing H-bonding interactions with AcOH between polypeptide side chains and NCA weakened the activation of monomers and slowed down the polymerization process.

Polymerization of nonpurified NCA with the acid-base equilibrium

The pK_a-dependent impact of organic acids on NCA polymerization allowed us to "turn on" NCA polymerization on demand because the inhibitory, stronger acid spontaneously turned into an accelerating, weaker acid upon the addition of the conjugate base of the weak acid (Figure 6a). The presence of strong organic acids like TFA enhanced the NCA stability against moisture, as the amino acid from the hydrolysis of NCA was unable to further polymerize other monomers. Indeed, the addition of water into the NCA/TFA mixture resulted in negligible degradation of NCA monomers (Supporting Information Figure S23). Therefore, it is possible to store NCA monomers premixed with initiators with the protection of TFA,

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

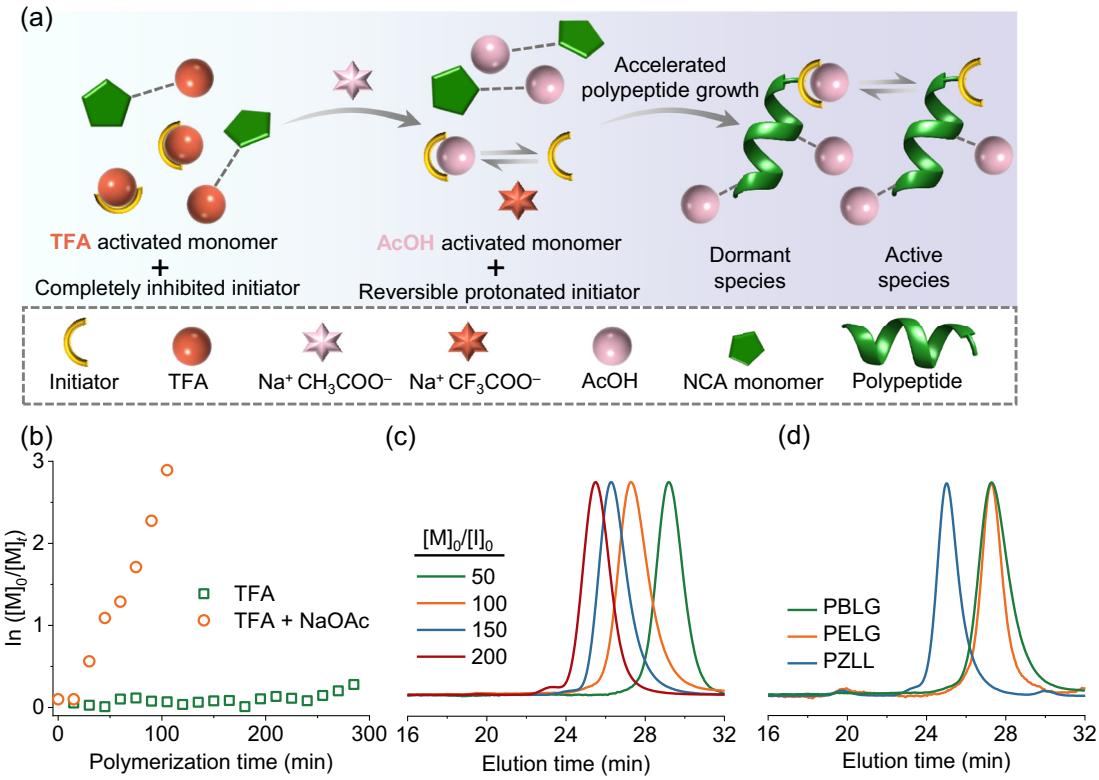


Figure 6 | *Polymerization of nonpurified NCA. (a) Scheme illustration showing the "turn on" process by converting the inhibitory acids into accelerating ones. (b) Semilogarithmic kinetic plot of polymerization of BLG-NCA initiated by Hex-NH_2 in DCM in the presence of TFA and TFA + NaOAc. $[M]_0/[I]_0/[TFA]_0$ = 100/1/100, $[M]_0 = [NaOAc]_0$ = 0.1 M. (c) Normalized GPC-LS traces of the resulting polypeptides obtained from the polymerization of nonpurified BLG-NCA at different $[M]_0/[I]_0$ ratios in the presence HCl and NaOAc. $[M]_0/[I]_0/[HCl]_0$ = 100/1/100, $[M]_0 = [NaOAc]_0$ = 0.1 M. (d) Normalized GPC-LS traces of the resulting polypeptides obtained in the presence of NaOAc with nonpurified BLG-NCA, ZLL-NCA and ELG-NCA as monomers. $[M]_0/[I]_0/[HCl]_0$ = 100/1/100, $[M]_0 = [NaOAc]_0$ = 0.1 M. NCA,* N*-carboxyanhydride; BLG-NCA, γ-benzyl-L-glutamate-*N*-carboxyanhydride; Hex-NH_2, n-hexylamine; DCM, dichloromethane; TFA, trifluoroacetic acid; NaOAc, sodium acetate; GPC-LS, gel permeation chromatography-light scattering; ZLL-NCA,* N$^{\varepsilon}$*-carboxybenzyl-L-lysine NCA; ELG-NCA, γ-ethyl-L-glutamate-NCA.*

which was then converted to accelerating, weaker acid to initiate the polymerization.

To verify our hypothesis, TFA was premixed with BLG-NCA at an equimolar ratio. No polymerization was observed 24 h after the addition of Hex-NH_2, substantiating the inhibitory effect of TFA. The addition of sodium acetate (NaOAc) converted the inhibitory effect of TFA into accelerating AcOH, where full NCA conversion was observed after 120 min (Figure 6b). The resulting polypeptides exhibited predictable MWs and low dispersity (Supporting Information Figure S24), demonstrating the robustness of AcOH-accelerated polymerization.

The acid-base equilibrium strategy was also used to convert HCl, one of the major impurities generated during NCA synthesis,[29,32,68] into accelerating AcOH during the direct polymerization of nonpurified NCA. Previous investigators relied on tedious and time-consuming anhydrous purification methods to remove impurities,[32,36] which are challenging to non-experts. Notably, nonpurified BLG-NCAs were obtained with a significantly higher isolation yield (>95%) compared to the 60% to ~80% yield achieved after purification. Additionally, this method allowed for a much shorter processing time (~2 h), as opposed to the days required for NCA purification. Thus, we envisaged that a strategy that could enable controlled polymerization of nonpurified NCA monomers would be of interest and impact. Chlorine analysis confirmed the existence of a trace amount of impurities, in which the chlorine content of nonpurified NCA was ~100 times that of purified NCA (Supporting Information Table S7). In the case of nonpurified BLG-NCAs, the presence of impurities such as HCl hindered the polymerization process when using a conventional polypeptide synthetic method. Upon mixing nonpurified BLG-NCAs with Hex-NH_2 in DCM, the NCA conversion was found to be less than 1% even after 12 h (Supporting Information Figure S25). While it was possible to remove HCl in situ through biphasic segregation, a sufficiently fast

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

polymerization was necessary to outpace water-induced side reactions.[29] The direct addition of NaOAc into the DCM solution of nonpurified NCA successfully eliminated the inhibitory effect of HCl but yielded ill-defined polypeptides (Supporting Information Figure S24), which was attributed to the initiation from the excessive acetate anions. Therefore, concentrated HCl was first added to the solution of nonpurified NCA, and then treated with NaOAc. PBLG end-capped with *n*-hexyl groups from nonpurified NCAs was obtained, resembling its counterpart from purified NCAs (Supporting Information Figure S26). Furthermore, polypeptides with various side chains and MWs were efficiently obtained with low dispersity (Figure 6c,d and Supporting Information Figure S27 and Table S8), substantiating the use of acid-base equilibrium to directly polymerize nonpurified NCA in a fast and controlled manner.

Conclusion

We report that the addition of organic acids, previously regarded as detrimental to the NCA polymerization process, enables the accelerated and controlled preparation of polypeptide materials. The acidity of the acids was critical to avoid the complete blocking of propagating chains while activating NCA monomers. The use of solvents with low polarity and weak H-bonding ability, such as DCM and chloroform, promoted molecular interactions and played an important role in uncovering the new role of acids in NCA polymerization. The current study highlights the possibility of altering the previous understanding of the NCA polymerization profile through a change in the polymerization conditions, inspiring the design of a new accelerating strategy.

Supporting Information

Supporting Information is available and includes materials, instrumentations, detailed experimental methods, NMR, FTIR, GPC, CD, MALDI-TOF data and calculated Gibbs free-energy profile (Figures S1–S27 and Tables S1–S8).

Conflict of Interest

There is no conflict of interest to report.

Preprint Statement

The research presented in this article was posted on a preprint server prior to publication in CCS Chemistry. The corresponding preprint article can be found here: (10.26434/chemrxiv-2023-906jv; https://chemrxiv.org/engage/chemrxiv/article-details/651cd2318bab5d20559a7044).

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC; grant nos. 52233015 for J. Cheng and 22101194 for Z. Song), the China Postdoctoral Science Foundation (grant nos. 2023M743133 for X. Liu and 2023M743140 for J. Huang), Natural Science Foundation of Jiangsu Province, China (grant no. BK20210733 for Z. Song), Suzhou Municipal Science and Technology Bureau (grant no. ZXL2021447 for Z. Song), Collaborative Innovation Center of Suzhou Nano Science & Technology, the 111 Project, Joint International Research Laboratory of Carbon-Based Functional Materials and Devices, and Suzhou Key Laboratory of Nanotechnology and Biomedicine, China. J. Cheng acknowledges the support of the New Cornerstone Investigator Program of the New Cornerstone Science Foundation, China. The authors thank Dr. Yinjuan Chen and Cuili Wang from Instrumentation and Service Center for Molecular Sciences at Westlake University, China, for supporting the chlorine analysis.

References

1. Kricheldorf, H. R. Polypeptides and 100 Years of Chemistry of α-Amino Acid *N*-Carboxyanhydrides. *Angew. Chem. Int. Ed.* **2006**, *45*, 5752–5784.
2. Leigh, T.; Fernandez-Trillo, P. Helical Polymers for Biological and Medical Applications. *Nat. Rev. Chem.* **2020**, *4*, 291–310.
3. Deming, T. J. Synthetic Polypeptides for Biomedical Applications. *Prog. Polym. Sci.* **2007**, *32*, 858–875.
4. Kataoka, K.; Harada, A.; Nagasaki, Y. Block Copolymer Micelles for Drug Delivery: Design, Characterization and Biological Significance. *Adv. Drug Deliv. Rev.* **2012**, *64*, 37–48.
5. He, C.; Zhuang, X.; Tang, Z.; Tian, H.; Chen, X. Stimuli-Sensitive Synthetic Polypeptide-Based Materials for Drug and Gene Delivery. *Adv. Healthcare Mater.* **2012**, *1*, 48–78.
6. Huang, J.; Heise, A. Stimuli Responsive Synthetic Polypeptides Derived from *N*-Carboxyanhydride (NCA) Polymerisation. *Chem. Soc. Rev.* **2013**, *42*, 7373–7390.
7. Deng, C.; Wu, J.; Cheng, R.; Meng, F.; Klok, H.-A.; Zhong, Z. Functional Polypeptide and Hybrid Materials: Precision Synthesis via α-Amino Acid *N*-Carboxyanhydride Polymerization and Emerging Biomedical Applications. *Prog. Polym. Sci.* **2014**, *39*, 330–364.
8. Song, Z.; Han, Z.; Lv, S.; Chen, C.; Chen, L.; Yin, L.; Cheng, J. Synthetic Polypeptides: From Polymer Design to Supramolecular Assembly and Biomedical Application. *Chem. Soc. Rev.* **2017**, *46*, 6570–6599.
9. Zhou, X.; Li, Z. Advances and Biomedical Applications of Polypeptide Hydrogels Derived from α-Amino Acid *N*-Carboxyanhydride (NCA) Polymerizations. *Adv. Healthcare Mater.* **2018**, *7*, 1800020.
10. Rasines Mazo, A.; Allison-Logan, S.; Karimi, F.; Chan, N. J.-A.; Qiu, W.; Duan, W.; O'Brien-Simpson, N. M.; Qiao,

G. G. Ring Opening Polymerization of α-Amino Acids: Advances in Synthesis, Architecture and Applications of Polypeptides and Their Hybrids. *Chem. Soc. Rev.* **2020**, *49*, 4737–4834.
11. Melnyk, T.; Đorđević, S.; Conejos-Sánchez, I.; Vicent, M. J. Therapeutic Potential of Polypeptide-Based Conjugates: Rational Design and Analytical Tools that Can Boost Clinical Translation. *Adv. Drug Deliv. Rev.* **2020**, *160*, 136–169.
12. Liu, Y.; Yin, L. α-Amino acid *N*-Carboxyanhydride (NCA)-Derived Synthetic Polypeptides for Nucleic Acids Delivery. *Adv. Drug Deliv. Rev.* **2021**, *171*, 139–163.
13. Deming, T. J. Facile Synthesis of Block Copolypeptides of Defined Architecture. *Nature* **1997**, *390*, 386–389.
14. Dimitrov, I.; Schlaad, H. Synthesis of Nearly Monodisperse Polystyrene–Polypeptide Block Copolymers via Polymerisation of *N*-Carboxyanhydrides. *Chem. Commun.* **2003**, 2944–2945.
15. Aliferis, T.; Iatrou, H.; Hadjichristidis, N. Living Polypeptides. *Biomacromolecules* **2004**, *5*, 1653–1656.
16. Lu, H.; Cheng, J. Hexamethyldisilazane-Mediated Controlled Polymerization of α-Amino Acid *N*-Carboxyanhydrides. *J. Am. Chem. Soc.* **2007**, *129*, 14114–14115.
17. Habraken, G. J. M.; Peeters, M.; Dietz, C. H. J. T.; Koning, C. E.; Heise, A. How Controlled and Versatile Is *N*-Carboxy Anhydride (NCA) Polymerization at 0 °C? Effect of Temperature on Homo-, Block- and Graft (Co)polymerization. *Polym. Chem.* **2010**, *1*, 514–524.
18. Liu, Y.; Li, D.; Ding, J.; Chen, X. Controlled Synthesis of Polypeptides. *Chin. Chem. Lett.* **2020**, *31*, 3001–3014.
19. Lv, W.; Wang, Y.; Li, M.; Wang, X.; Tao, Y. Precision Synthesis of Polypeptides via Living Anionic Ring-Opening Polymerization of *N*-Carboxyanhydrides by Tri-thiourea Catalysts. *J. Am. Chem. Soc.* **2022**, *144*, 23622–23632.
20. Zou, J.; Fan, J.; He, X.; Zhang, S.; Wang, H.; Wooley, K. L. A Facile Glovebox-Free Strategy to Significantly Accelerate the Syntheses of Well-Defined Polypeptides by *N*-Carboxyanhydride (NCA) Ring-Opening Polymerizations. *Macromolecules* **2013**, *46*, 4223–4226.
21. Zhao, W.; Gnanou, Y.; Hadjichristidis, N. Organocatalysis by Hydrogen-Bonding: A New Approach to Controlled/Living Polymerization of α-Amino acid *N*-Carboxyanhydrides. *Polym. Chem.* **2015**, *6*, 6193–6201.
22. Baumgartner, R.; Fu, H.; Song, Z.; Lin, Y.; Cheng, J. Cooperative Polymerization of α-Helices Induced by Macromolecular Architecture. *Nat. Chem.* **2017**, *9*, 614–622.
23. Yuan, J.; Zhang, Y.; Li, Z.; Wang, Y.; Lu, H. A S-SN Lewis Pair-Mediated Ring-Opening Polymerization of α-Amino Acid *N*-Carboxyanhydrides: Fast Kinetics, High Molecular Weight, and Facile Bioconjugation. *ACS Macro Lett.* **2018**, *7*, 892–897.
24. Wu, Y.; Zhang, D.; Ma, P.; Zhou, R.; Hua, L.; Liu, R. Lithium Hexamethyldisilazide Initiated Superfast Ring Opening Polymerization of Alpha-Amino Acid *N*-Carboxyanhydrides. *Nat. Commun.* **2018**, *9*, 5297.
25. Jacobs, J.; Pavlović, D.; Prydderch, H.; Moradi, M.-A.; Ibarboure, E.; Heuts, J. P. A.; Lecommandoux, S.; Heise, A. Polypeptide Nanoparticles Obtained from Emulsion Polymerization of Amino Acid *N*-Carboxyanhydrides. *J. Am. Chem. Soc.* **2019**, *141*, 12522–12526.
26. Zhao, W.; Lv, Y.; Li, J.; Feng, Z.; Ni, Y.; Hadjichristidis, N. Fast and Selective Organocatalytic Ring-Opening Polymerization by Fluorinated Alcohol Without a Cocatalyst. *Nat. Commun.* **2019**, *10*, 3590.
27. Zhao, W.; Lv, Y.; Li, J.; Feng, Z.; Ni, Y.; Hadjichristidis, N. A Synthetic Method for Site-Specific Functionalized Polypeptides: Metal-Free, Highly Active, and Selective at Room Temperature. *Angew. Chem. Int. Ed.* **2021**, *60*, 889–895.
28. Wu, Y.; Chen, K.; Wu, X.; Liu, L.; Zhang, W.; Ding, Y.; Liu, S.; Zhou, M.; Shao, N.; Ji, Z.; Chen, J.; Zhu, M.; Liu, R. Superfast and Water-Insensitive Polymerization on α-Amino Acid *N*-Carboxyanhydrides to Prepare Polypeptides Using Tetraalkylammonium Carboxylate as the Initiator. *Angew. Chem. Int. Ed.* **2021**, *60*, 26063–26071.
29. Song, Z.; Fu, H.; Wang, J.; Hui, J.; Xue, T.; Pacheco, L. A.; Yan, H.; Baumgartner, R.; Wang, Z.; Xia, Y.; Wang, X.; Yin, L.; Chen, C.; Rodríguez-López, J.; Ferguson, A. L.; Lin, Y.; Cheng, J. Synthesis of Polypeptides via Bioinspired Polymerization of In Situ Purified *N*-Carboxyanhydrides. *Proc. Natl. Acad. Sci.* **2019**, *116*, 10658–10663.
30. Grazon, C.; Salas-Ambrosio, P.; Ibarboure, E.; Buol, A.; Garanger, E.; Grinstaff, M. W.; Lecommandoux, S.; Bonduelle, C. Aqueous Ring-Opening Polymerization-Induced Self-Assembly (ROPISA) of *N*-Carboxyanhydrides. *Angew. Chem. Int. Ed.* **2020**, *59*, 622–626.
31. Hu, Y.; Tian, Z.-Y.; Xiong, W.; Wang, D.; Zhao, R.; Xie, Y.; Song, Y.-Q.; Zhu, J.; Lu, H. Water-Assisted and Protein-Initiated Fast and Controlled Ring-Opening Polymerization of Proline *N*-Carboxyanhydride. *Natl. Sci. Rev.* **2022**, *9*, nwac033.
32. Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. Synthesis of Well-Defined Polypeptide-Based Materials via the Ring-Opening Polymerization of α-Amino Acid *N*-Carboxyanhydrides. *Chem. Rev.* **2009**, *109*, 5528–5578.
33. Kramer, J. R.; Deming, T. J. General Method for Purification of α-Amino Acid-*N*-Carboxyanhydrides Using Flash Chromatography. *Biomacromolecules* **2010**, *11*, 3668–3672.
34. Fuchs, F. Über *N*-Carbonsäure-Anhydride. *Ber. Dtsch. Chem. Ges.* **1922**, *55*, 2943–2943.
35. Farthing, A. C.; Reynolds, R. J. W. Anhydro-*N*-Carboxy-DL-β-Phenylalanine. *Nature* **1950**, *165*, 647–647.
36. Kricheldorf, H. R. Oligomerization and Polymerization of NCAs: Chemical Aspects. In *α-Amino Acid-N-Carboxy-Anhydrides and Related Heterocycles: Syntheses, Properties, Peptide Synthesis, Polymerization*;Kricheldorf, H. R., Ed.; Springer: Berlin, Heidelberg, **1987**; pp 59–157.
37. Zelzer, M.; Heise, A. Determination of Copolymerisation Characteristics in the *N*-Carboxy Anhydride Polymerisation of Two Amino Acids. *Polym. Chem.* **2013**, *4*, 3896–3904.
38. Koch, S. A.; Doyle, T. D. Direct Determination of Amine Salt-Base Ratios by Nuclear Magnetic Resonance Spectrometry: Correlation of Acid Strengths in Chloroform by Nuclear Magnetic Resonance and Infrared Spectrometry. *Anal. Chem.* **1967**, *39*, 1273–1276.

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954

39. Conejos-Sánchez, I.; Duro-Castano, A.; Birke, A.; Barz, M.; Vicent, M. J. A Controlled and Versatile NCA Polymerization Method for the Synthesis of Polypeptides. *Polym. Chem.* **2013**, *4*, 3182–3186.
40. Li, L.; Cen, J.; Pan, W.; Zhang, Y.; Leng, X.; Tan, Z.; Yin, H.; Liu, S. Synthesis of Polypeptides with High-Fidelity Terminal Functionalities Under NCA Monomer-Starved Conditions. *Research* **2021**, *2021*, 9826046.
41. Kamber, N. E.; Jeong, W.; Waymouth, R. M.; Pratt, R. C.; Lohmeijer, B. G. G.; Hedrick, J. L. Organocatalytic Ring-Opening Polymerization. *Chem. Rev.* **2007**, *107*, 5813–5840.
42. Dove, A. P. Metal-Free Catalysis in Ring-Opening Polymerization. In *Handbook of Ring-Opening Polymerization*; Dubois, P., Coulembier, O., Raquez, J.-M., Eds.; Weinheim: Wiley-VCH, **2009**; pp 357–378.
43. Kiesewetter, M. K.; Shin, E. J.; Hedrick, J. L.; Waymouth, R. M. Organocatalysis: Opportunities and Challenges for Polymer Synthesis. *Macromolecules* **2010**, *43*, 2093–2107.
44. Ling, J.; Huang, Y. Understanding the Ring-Opening Reaction of α-Amino Acid *N*-Carboxyanhydride in an Amine-Mediated Living Polymerization: A DFT Study. *Macromol. Chem. Phys.* **2010**, *211*, 1708–1711.
45. Thomas, C.; Bibal, B. Hydrogen-Bonding Organocatalysts for Ring-Opening Polymerization. *Green Chem.* **2014**, *16*, 1687–1699.
46. Xu, J.; Wang, X.; Liu, J.; Feng, X.; Gnanou, Y.; Hadjichristidis, N. Ionic H-Bonding Organocatalysts for the Ring-Opening Polymerization of Cyclic Esters and Cyclic Carbonates. *Prog. Polym. Sci.* **2022**, *125*, 101484.
47. Sehgal, D.; Vijay, I. K. A Method for the High Efficiency of Water-Soluble Carbodiimide-Mediated Amidation. *Anal. Biochem.* **1994**, *218*, 87–91.
48. Albert, A.; Serjeant, E. P. The Ionization Constants of Typical Acids and Bases. In *The Determination of Ionization Constants: A Laboratory Manual*;Albert, A., Serjeant, E. P., Eds.; Springer Netherlands: Dordrecht, **1984**; pp 136–175.
49. Ballard, D. G. H.; Bamford, C. H.; Wilson, A. H. Studies in Polymerization - VII. The Polymerization of N-Carboxy-α-Amino Acid Anhydrides. *Proc. R. Soc. Lond. A* **1997**, *223*, 495–520.
50. Siefker, D.; Williams, A. Z.; Stanley, G. G.; Zhang, D. Organic Acid Promoted Controlled Ring-Opening Polymerization of α-Amino Acid-Derived *N*-Thiocarboxyanhydrides (NTAs) Toward Well-Defined Polypeptides. *ACS Macro Lett.* **2018**, *7*, 1272–1277.
51. Zheng, B.; Xu, S.; Ni, X.; Ling, J. Understanding Acid-Promoted Polymerization of the *N*-Substituted Glycine *N*-Thiocarboxyanhydride in Polar Solvents. *Biomacromolecules* **2021**, *22*, 1579–1589.
52. Zhou, P.; Shen, T.; Chen, W.; Sun, J.; Ling, J. Biodegradable Polysarcosine with Inserted Alanine Residues: Synthesis and Enzymolysis. *Biomacromolecules* **2022**, *23*, 1757–1764.
53. Yu, X.; Wang, Y.; Dong, Y.; Zhao, N.; Zhang, L.; Xuan, S.; Zhang, Z. Efficient Synthesis of *N*-Methyl Polypeptides by Organic Acid Promoted Controlled Ring-Opening Polymerization of *N*-Methyl-α-Amino Acids *N*-Carboxyanhydrides. *Macromolecules* **2023**, *56*, 8899–8911.
54. Wang, S.; Lu, H. Ring-Opening Polymerization of Amino Acid *N*-Carboxyanhydrides with Unprotected/Reactive Side Groups. I. D-Penicillamine *N*-Carboxyanhydride. *ACS Macro Lett.* **2023**, *12*, 555–562.
55. Song, Z.; Fu, H.; Baumgartner, R.; Zhu, L.; Shih, K.-C.; Xia, Y.; Zheng, X.; Yin, L.; Chipot, C.; Lin, Y.; Cheng, J. Enzyme-Mimetic Self-Catalyzed Polymerization of Polypeptide Helices. *Nat. Commun.* **2019**, *10*, 5470.
56. Chen, C.; Fu, H.; Baumgartner, R.; Song, Z.; Lin, Y.; Cheng, J. Proximity-Induced Cooperative Polymerization in "Hinged" Helical Polypeptides. *J. Am. Chem. Soc.* **2019**, *141*, 8680–8683.
57. Lv, S.; Kim, H.; Song, Z.; Feng, L.; Yang, Y.; Baumgartner, R.; Tseng, K.-Y.; Dillon, S. J.; Leal, C.; Yin, L.; Cheng, J. Unimolecular Polypeptide Micelles via Ultrafast Polymerization of *N*-Carboxyanhydrides. *J. Am. Chem. Soc.* **2020**, *142*, 8570–8574.
58. Wang, W.; Fu, H.; Lin, Y.; Cheng, J.; Song, Z. Cooperative Covalent Polymerization of *N*-Carboxyanhydrides: From Kinetic Studies to Efficient Synthesis of Polypeptide Materials. *Acc. Mater. Res.* **2023**, *4*, 604–615.
59. Xia, Y.; Song, Z.; Tan, Z.; Xue, T.; Wei, S.; Zhu, L.; Yang, Y.; Fu, H.; Jiang, Y.; Lin, Y.; Lu, Y.; Ferguson, A. L.; Cheng, J. Accelerated Polymerization of *N*-Carboxyanhydrides Catalyzed by Crown Ether. *Nat. Commun.* **2021**, *12*, 732.
60. Li, Q.; Lan, Y.; Wang, W.; Ji, G.; Li, X.; Song, Z. Polymerization of *N*-Carboxyanhydride in Cosolvents: The Balance Between the Polymerization Rate and Molecular Weight Control. *Macromolecules* **2023**, *56*, 7023–7031.
61. Walvoord, R. R.; Huynh, P. N. H.; Kozlowski, M. C. Quantification of Electrophilic Activation by Hydrogen-Bonding Organocatalysts. *J. Am. Chem. Soc.* **2014**, *136*, 16055–16065.
62. Nederberg, F.; Connor, E. F.; Möller, M.; Glauser, T.; Hedrick, J. L. New Paradigms for Organic Catalysts: The First Organocatalytic Living Polymerization. *Angew. Chem. Int. Ed.* **2001**, *40*, 2712–2715.
63. Steiner, T. The Hydrogen Bond in the Solid State. *Angew. Chem. Int. Ed.* **2002**, *41*, 48–76.
64. Bianchi, G.; Howarth, O. W.; Samuel, C. J.; Vlahov, G. Long-Range σ-Inductive Interactions Through Saturated C–C Bonds in Polymethylene Chains. *J. Chem. Soc., Perkin Trans. 2* **1995**, *116*, 1427–1432.
65. Bowden, K.; Grubbs, E. J. Through-Bond and Through-Space Models for Interpreting Chemical Reactivity in Organic Reactions. *Chem. Soc. Rev.* **1996**, *25*, 171–177.
66. Lohmeijer, B. G. G.; Pratt, R. C.; Leibfarth, F.; Logan, J. W.; Long, D. A.; Dove, A. P.; Nederberg, F.; Choi, J.; Wade, C.; Waymouth, R. M.; Hedrick, J. L. Guanidine and Amidine Organocatalysts for Ring-Opening Polymerization of Cyclic Esters. *Macromolecules* **2006**, *39*, 8574–8583.
67. Anderson, W. R.; Silverstein, R. M. Structure of Amines by Nuclear Magnetic Resonance Spectrometry. *Anal. Chem.* **1965**, *37*, 1417–1418.
68. Tian, Z.; Zhang, Z.; Wang, S.; Lu, H. A Moisture-Tolerant Route to Unprotected α/β-Amino Acid *N*-Carboxyanhydrides and Facile Synthesis of Hyperbranched Polypeptides. *Nat. Commun.* **2021**, *12*, 5810.

DOI: 10.31635/ccschem.024.202403954
Citation: *CCS Chem.* **2025**, 7, 2769–2780
Link to VoR: https://doi.org/10.31635/ccschem.024.202403954