A New Synthetic Methodology of Site-Specific Functionalized Polypeptides: Metal-Free, Highly Active and Selective at Room Temperature

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Abstract: Functionalized polypeptides have attracted tremendous interest in recent years and found many stimulating applications due to their tunable physicochemical characteristics including hydrophilicity and stimuli-responsive behavior. The development of new strategies to produce these polymers without metallic contaminants is crucial for their applications in high-value and sensitive domains, such as biomedical, microelectronic, food packaging and personal beauty care fields. Herein, a highly efficient strategy to access well-defined site-specific functionalized polypeptides is developed by combining Michael reaction with hydrogen-bonding organocatalytic ROP of NCA. A library of chain-end and chain-middle functionalized polypeptides (14 examples) with predesigned molecular weights and low polydispersities are readily prepared with this approach. Specifically, the whole synthetic process is metal-free, fulfilling high activity and selectivity at room temperature.

Introduction

Polymers are ubiquitous and highly usefully materials in modern society. The development of new strategies to prepare polymer materials for people's daily needs has been the eternal theme of polymer science.^[1-2] In recent years, scientific efforts towards design, synthesis, and production of environmentally friendly and sustainable polymers have gained more and more attention.^[3] As for sustainable polymers such as polylactones, polylactides, polycarbonates and polypeptides, a green synthetic process is particularly crucial for their applications in high-value and sensitive domains, such as biomedical, microelectronic, food packaging and personal beauty care fields. In this context, exploration of new methods to produce these polymers without metallic contaminants has led to a continuing interest in metal-free controlled/living polymerization.^[4-8]

Inspired by the rapid progress of metal-free organic transformations,^[9] scientists have explored the applications of metal-free catalytic systems for ROP of cyclic esters (lactide, lactones, phosphoesters) and carbonates to prepare sustainable polymers.^[6] Since the first report of organocatalytic living ROP of

lactide by Hedrick *et al.* in 2001,^[10] strongly basic amines, *N*-heterocyclic carbenes (NHCs),^[11-13] bifunctional thiourea/R₃N systems^[14] and ionic (thio)ureas^[15] have been investigated for controlled/living ROP of cyclic esters/carbonates. These compounds have wide applications in organic chemistry for various organic transformations.^[16,17] Especially, the bifunctional thiourea/R₃N systems explored for ROP by Waymouth *et al.* in 2005,^[14] in which a general base motif (R₃N) was used to activate the ROH initiator/chain-end and a thiourea to activate the monomer and stabilize the incipient charged tetrahedral intermediate, have been widely used for the synthesis of sustainable polymer materials without metallic contaminants. Very recently, an elegant bicomponent metal-free system based on triethylborane (Et₃B)/phosphazene base (tBuP₂) was developed by Zhao, Liu, and co-workers for the copolymerization of cyclic ethers and lactones. By precisely controlling the Lewis acid (Et₃B)/base (tBuP₂) catalysts ratio, the propagating active species can selectively incorporate cyclic ethers or lactones to polymer chains, resulting in clean ether-ester block copolymers with unlimited sequences.^[18] Therefore, as an alternative to organometallic and enzymatic catalysis, organocatalysis provides promising approaches to the preparation of clean polymers.

Synthetic polypeptides as mimics of natural analogs are a class of bio-inspired sustainable materials with broad applications in biomedical fields such as drug release, gene delivery, tissue engineering, and regenerative medicines.^[19,20] Ring-opening polymerization (ROP) of α -amino acid *N*-carboxyanhydrides (NCAs) initiated by different amines/amine derivatives is the most common method used to access synthetic polypeptide. However, the polymerization reactions usually are very sluggish and uncontrollable, resulting in poor control of the polymer microstructures.^[21,22] Since the first achievement of living ROP of NCAs by Deming in 1997,^[23] a series of metal based/metal-free initiators such as Pt complexes,^[24] ammonium salts,^[25] silazane derivatives,^[26] rare earth metal complexes,^[27] and amine-borane Lewis pairs^[28] were reported for the controlled NCA polymerizations. Despite the excellent control over molecular weights and molecular weight distributions, the polymerization rates are usually slow. Thanks to the continuous contributions and efforts of researchers over the last few years, this situation has been improved and several new initiating systems such as allied amines,^[29] *N*-trimethylsilyl amines,^[30] sulfur-tin Lewis pair,^[31] *N*-heterocyclic carbenes (NHCs),^[32] aliphatic diamines^[33] and lithium hexamethyldisilazide,^[34] and new organocatalytic systems based on organic hydrogen-bonding donors^[35,36] were successively reported for ROP of NCAs with high polymerization selectivities and fast kinetics, which greatly facilitated the access of polypeptide materials. However, few of these new systems have been explored for the synthesis of functionalized polypeptides. Currently, the mainly used method to access functionalized polypeptides especially site-specific functionalized polypeptides

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still relies on the ROPs of NCAs initiated by primary amine derivatives and the precise control over the microstructures of the final materials is still challenging.^[37]

In this contribution, we report a new, simple, and metal-free methodology for the synthesis of well-defined site-specific functionalized polypeptides. The new concept takes advantage of the highly efficient Michael reactions between acrylates and secondary-aminoalcohols, allowing facile preparation of a family of functionalized tertiary-aminoalcohols (14 examples) with quantitative yields. In cooperation with Schreiner thiourea (**TU-S**), a organocatalyst for organic transformations,^[38] all of the resultant tertiary aminoalcohols can successfully trigger ROP of NCAs, leading to a variety of site-specific functionalized polypeptides with narrow molecular weight distributions and designed molecular weights. The functionalized groups can be readily anchored at the chain-end or in the middle of the resultant polypeptides (Scheme 1). Specially, the whole polymerization process is metal-free, highly active and selective at room temperature. The new strategy provides an easy way to obtain clean functionalized polypeptides for researchers who focus on applications of polypeptide-based materials but have backgrounds other than polymer synthesis.

Scheme 1. A new strategy investigated in this study for site-specific functionalized polypeptide synthesis. (Structure lines (bonds) thicker)

Results and Discussion

In our pursuit of exploring highly active and selective metal-free catalytic systems for polypeptide synthesis,^[29,35,36] we found that organic hydrogen-bonding donors, such as thiourea and fluorinated alcohol, could function as efficient organocatalysts for the ROP of *N*-carboxyanhydride (NCA).^[35,36] Different from the “bicomponent-bifunctional” catalytic systems (thiourea/ R_3N) for ROPs of cyclic esters, the hydrogen-bonding donor, thiourea or fluorinated alcohol, can catalyze ROP of NCAs in the absence of cocatalysts (usually weak bases), demonstrating an unconventional “monocomponent-multifunctional” catalytic mode. During polymerization, the catalyst alone plays a multiplying role via cooperative hydrogen-bonding interactions with monomer, initiator and chain active species, which activates the NCA monomer and simultaneously suppresses the side reactions such as chain-transfer and chain-termination, rendering the whole polymerization highly active and selective at room temperature.

In the presence of these hydrogen-bonding donor catalysts, a series of commercially available tertiary-aminoalcohols such as **DMEA**, **MDEA**, **TEA**, and **THEED**, can be used as initiators for the ROP of NCA monomers, producing polypeptides with tunable molecular topologies (Scheme 2). However, all of these tertiary-aminoalcohols have inert molecular structures, in which the only reactive nitrogen atoms are fully “occupied” by the substituent groups and has no vacant reactive sites. Therefore, it is challenging to incorporate functional groups or other polymerization initiating sites into these compounds to generate functional or hetero-bifunctional Initiators and then synchronize with hydrogen-bonding donor-based catalyst to prepare polypeptides with specific functions or polypeptide-hybrid copolymers. Since the initiator usually is covalently attached at polymer chain-ends after polymerization and becomes an integral part of the whole polymer chain, the chemical structure nature of the initiator can affect the composition, functionality and topology of the final polymer and thus the polymer properties. Deficient functionalization of initiator for the ROP of NCA will largely limit the access to more varieties of polypeptide-based materials^[1].

Scheme 2. Commercially available tertiary-aminoalcohols used for topology control of polypeptide.

Scheme 3. The difference of ROP of NCA initiated by secondary and tertiary aminoalcohols in the presence of Schreiner thiourea (**TU-S**).

We previously found that beta-aminoalcohols such as *N*-methylmonoethanolamine (**MMEA**) and *N,N*-dimethylethanolamine (**DMEA**) could function as initiators for the ROP of *N*-carboxyanhydride (NCA) in the presence of Schreiner thiourea (**TU-S**) catalyst, but common alcohol compounds such as isopropanol, benzyl alcohol, and tert-butyl alcohol could not.^[35] This is because the nitrogen atom in aminoalcohol can form intramolecular hydrogen-bonding with the hydroxyl group, thus increasing the nucleophilicity of the oxygen atom. This hydrogen-bonding interaction can promote the nucleophilic attack of hydroxyl group to carbonyl of NCA monomer, resulting in the initiating of polymerization (Scheme 3). However, the polymerization behaviors of NCA monomers largely depend on the kind of the aminoalcohol initiators employed.^[35] In the case of tertiary-aminoalcohols such as **DMEA** used as an initiator, the polymerization was fast and selective, giving well-defined

polypeptides. As for secondary-aminoalcohol initiators such as **MMEA**, the polymerization could be triggered but was slow and unselective, leading to polypeptides with broad molecular weight distributions and higher molecular weights than designed ones, indicating an unwanted low initiation rate (vs. propagating rate). The poor performance of polymerization initiated by secondary aminoalcohols probably is caused by a slow initiating reaction due to the weaker nucleophilicity of the hydroxyl group in the secondary-aminoalcohol (vs. that in tertiary-aminoalcohol) (Scheme 3).

Inspired by the very different behaviours of the polymerizations caused by the subtle structural difference of the aminoalcohols initiators, we propose a new strategy shown in Scheme 4 to transform the secondary aminoalcohol **MMEA** into tertiary one via Michael reaction between the acrylate and the secondary amine group, with the aim to incorporate functionalized groups (from acrylates), simultaneously, at the beta-position of hydroxyl groups in final aminoalcohols. Since there are many commercially available acrylates, a variety of functionalized tertiary aminoalcohols can easily be accessed and then combined with organocatalysts for the ROP of NCAs to prepare a library of clean functionalized polypeptides.

We first studied the synthesis of initiator **1a** from ethyl acrylate (**a**) and *N*-methylmonoethanolamine (**MMEA**). The reaction between **a** and **MMEA** was very fast and clean, as proved by *in situ* FT-IR and NMR spectra (Figure 1 and Figures S1-S4). We obtained initiator **1a** in a quite simple way. 1.1 equiv. **a** was added to 1 equiv. **MMEA** and the solvent-free mixture was stirred at room temperature for 10 minutes. The excess **a** was then removed in vacuum, resulting in **1a** as a colorless oil with quantitative yield. We examined the initiating capability of **1a** for ROP of NCA in the presence of Schreiner thiourea (**TU-S**). A mixture of **1a** and 2 equiv. **TU-S** was suspended in dichloromethane and treated with 120 equiv. NCA monomer. The polymerization is fast and all NCA monomers could be converted to polypeptide in 35 minutes at room temperature. The polymerization progress was further monitored by using *in situ* FTIR at fixed time intervals (10 seconds) for a minimum of four half-life times. As shown in Figure 2 (black square line), the plot of $\ln([M]_0/[M]_t)$ vs. time shows two successive straight lines with different slopes, which is due to the secondary structure transition of PBLG from non-helices to α -helices (when the DP is reached to 7–13).^[39,40] The linearity of the two lines suggests that the initiation is faster or comparable to chain propagation under the experimental conditions. A high apparent polymerization rate constant (k_{app}) up to 11.30 h^{-1} was obtained from the slope of the fitted line. The polymerization also retained high selectivity of the NCA ring-opening reactions over other competing ones (chain termination and chain transfer reactions), resulting in polypeptide with designed M_n and a very narrow molecular weight distribution (Table 1, run 1, Figure S5a). Detailed kinetics shows that the molecular weight (M_n) of polypeptide increases linearly with monomer conversion and the monomers can be completely consumed with monomer conversion as high as 99%. the SEC traces of samples taken during polymerization were

narrow and symmetric, with $\bar{D}$ values below 1.06 (Figure S6). In addition, the polymerization continued smoothly when additional monomers were added into the system, resulting in a unimodal polymer (Figure S7). The end group analyses of a low molecular weight sample, synthesized under similar conditions by using ^1H NMR spectroscopy further support the controlled nature of the polymerization. The resonances of **1a** could be clearly observed in the ^1H NMR spectrum, and the experimental M_n calculated based on NMR spectrum was very close to the theoretical value (Figure 3). Therefore, the whole polymerization possesses all characteristic features of "livingness": (a) linearity of M_n with conversion; (b) complete consumption of the monomer; (c) excellent control of molecular weight and narrow molecular weight distributions; and (e) continuing and resulting in unimodal polymer after new addition of monomers.^[41]

Scheme 4. Synthesis of chain-end functionalized polypeptide via Michael reaction and organocatalytic ROP of NCA.

We further prepared **1b–1g** by using a method similar to **1a** from the reactions of **b–g** and **MMEA** (Scheme 4). To our delight, all reactions were fast and gave target products with high yields. In the case of acrylates with high boiling temperatures, we could easily remove the excess acrylates by simply washing the products with diethyl ether (the ^1H and ^{13}C NMR spectra of **1b–1g** see Figures S8–S19). As expected, with a 0.8 mol% loading

of **TU-S** catalyst relative to Glu-NCA (**TU-S/1b-1g**=2, $M/I=120$), all obtained functionalized initiators could trigger the ROP of NCAs efficiently in DCM at room temperature. The NCA monomers could be converted into polypeptides in 75 minutes. In addition, all polymerizations showed high tolerance against functional groups and demonstrated similar kinetics behaviours to those initiated by **1a**. A first-order dependence on the monomer concentration was observed and the plot of $\ln([M]_0/[M]_t)$ vs. time gave two successive straight lines with different slopes (Figures 2, S20-S26), suggesting fast initiating of the polymerizations (vs. chain propagation) under the experimental conditions. Moreover, all obtained polymers exhibited very narrow symmetric SEC traces ($PDI < 1.05$, Figures S5b-g), and the molecular weights M_n were close to the theoretical ones (Table 1, runs 2-7), producing a series of chain-end functionalized polypeptides with well-defined structures.

Figure 1. In situ FT-IR monitoring of the reaction between **1a** and **MMEA** (conditions: $[1a]/[MMEA]=1.1$, 25°C, no solvent).

Table 1. Controlled ROP of Glu-NCA initiated by functionalized tertiary-aminoalcohol initiators in the presence of **TU-S** catalyst.

Run ^[a]	I	$[M]_0/[I]_0$ /[TU-S]	Time (min) ^[b]	$M_{n,calc}$ $\times 10^{-4}$ [c]	$M_{n,SEC-LS}$ $\times 10^{-4}$ [d]	PDI [d]
1	1a	120/1/2	35	2.63	2.30	1.06
2	1b	120/1/2	40	2.63	2.67	1.03
3	1c	120/1/2	55	2.63	2.52	1.05
4	1d	120/1/2	70	2.63	2.81	1.04
5	1e	120/1/2	75	2.63	2.86	1.03
6	1f	120/1/2	60	2.63	2.90	1.03
7	1g	120/1/2	45	2.63	2.70	1.05
8	2a	120/1/2	50	2.63	2.87	1.04
9	2b	120/1/2	55	2.63	2.79	1.05
10	2c	120/1/2	80	2.63	2.24	1.08
11	2d	120/1/2	60	2.63	2.71	1.06
12	2e	120/1/2	75	2.63	2.56	1.09
13	2f	120/1/2	120	2.63	3.03	1.09
14	2g	120/1/2	95	2.63	2.80	1.08

[a] Polymerization was carried out in dichloromethane at 25°C with $[NCA]_0 = 0.19M$. [b] In situ FT-IR was used to determine the time for full monomer conversion by analysing the intensity of the NCA anhydride absorption band at 1792 cm^{-1} . [c] Calculated by $[NCA]/[I] \times (M_{NCA}-44) \times X$ ($X = \text{Conv.}$). [d] Determined by SEC-MALS, 0.1 M LiBr in DMF at 60 °C.

Figure 2. Kinetics of the ROP of Glu-NCA promoted by **1a-1g/TU-S**, conditions: $[Glu-NCA]/[1a-1g]/[TU-S]=120/1/2$, $[M] = 0.19\text{ M}$, 25°C, CH_2Cl_2 , the automatic sampling interval of *in situ* FT-IR is 10 seconds (The k_{app} values were obtained from the linear fits of the plots. The individual plots of $\ln([NCA]_0/[NCA]_t)$ vs. time and corresponding linear fits see Figures S20-S26).

Figure 3. 1H NMR spectrum of PBLG ($DP=5$) from polymerization initiated by **1a/TU-S** (400M, 25°C, $CDCl_3/CF_3COOD$ (2:1), the theoretical M_n is 1096 and the $M_{n,NMR}$ calculated from NMR spectrum is 1315).

We performed computational studies of **MMEA**, **DMEA** and the resultant aminoalcohols **1a-1g** at the B3LYP/6-311G level of theory (Figure 4). All of structures are fully optimized and electrostatic potential (ESP)-fitting charges are derived from the DFT calculation. The lowest-energy structure of **DMEA** ($\Delta E = -19.305\text{ kcal/mol}$) demonstrates that an intramolecular hydrogen bond is formed between the hydroxyl and tertiary amine group ($O-H \cdots N(CH_3)_2$) in **DMEA**. The charges assigned to the oxygen of the hydroxyl group and nitrogen atoms are -0.600 and -0.104, respectively. In the minimum energy structure of **MMEA** ($\Delta E = -20.598\text{ kcal/mol}$), the hydroxyl group can also form hydrogen-bonds with the secondary amine ($O-H \cdots NHCH_3$). The charge assigned to the oxygen in **MMEA** is higher than that in the **DMEA** (-0.585 vs. -0.600), suggesting a lower nucleophilicity of the hydroxyl group in **MMEA** than that of **DMEA**. At the same time, the charge of the nitrogen atom in **MMEA** is much lower than that of **DMEA** (-0.584 vs. -0.104) but comparable to that of

the oxygen atom in **MMEA**, which indicate that the secondary amine in **MMEA** may also function as an additional initiating species for the ROP of NCA. The existence of two different kinds of active species will lead to poor control over the ROP of NCAs. These results from the computational analysis are consistent with the polymerization behaviours observed in the ROP of NCA initiated by **DMEA** and **MMEA**, respectively. Under identical conditions, the **DMEA** initiates a fast and selective ROP of NCA, while **MMEA** triggers a slow and unselective one. By comparing the lowest energy structures of the resultant aminoalcohols **1a-1g**, we find that hydrogen bonds are also formed between the hydroxyl and tertiary amine groups ($\text{O}\cdots\text{NRCH}_3$) in these new tertiary-aminoalcohols (Figure 5 and Figures S27-35). The charges assigned to the oxygen atoms have similar values ranging from -0.633 to -0.612, which are comparable to that of **DMEA** (-0.600) but higher than that of **MMEA** (-0.585). These results are consistent with the similar polymerization behaviours observed from the ROP of NCA by using these functionalized aminoalcohols.

Figure 4. Relative energies of different configurations of DMEA and MMEA according to DFT calculation, (a) **DMEA** and (b) **MMEA**.

To further extend this strategy to the preparation of chain-middle functionalized polypeptides, we investigated the reactions of **a-g** with diethanolamine(**DEA**) to prepare functionalized initiators **2a-2g** (Scheme 5). Since the secondary nitrogen atom of **DEA** is located in the middle of the molecule,

after reactions with the acryl groups of **a-g** the functionalized group from acrylates will be anchored at the nitrogen atoms. If the resultant aminoalcohols are used as initiators for ROP of NCA, the functionalized group will be incorporated at the middle of the chain after chain propagating from the two end-hydroxyls of **2a-2g**, resulting in chain-middle functionalized polypeptides.

Figure 5. Lowest energy structures of **MMEA**, **DMEA** and functionalized tertiary aminoalcohol **1a-1g**.

Compared with the reaction between **a-g** and **MMEA**, the reactions between **a-g** and **DEA** were slower due to the higher steric hindrance of **DEA**, but we still could get target functionalized aminoalcohols (**2a-2g**) with quantitative yields in one hour by elevating the reaction temperature to 50°C (the ^1H and ^{13}C NMR spectra of **2a-2g** see Figures S36-S49). In the presence of **TU-S** catalyst, all functionalized aminoalcohol (**2a-2g**) can successfully trigger the ROP of NCA and a series of chain-middle functionalized polypeptides was readily prepared with high activity and selectivity. The NCA monomers can be completely converted into the polymer in 120 minutes at room

temperature, and the resultant polypeptides possess predesigned molecular weights and narrow molecular weight distributions (Table 1, Runs 8-14, Figure S50). The polymerization kinetics is similar to that of the ROP of NCA initiated by **1a-1g** (Figures 6, S51-57). A first-order dependence on NCA monomer concentration was observed. The plot of $\ln([M]_0/[M]_t)$ vs. time gave two successive straight lines with different slopes, indicating fast chain initiating of polymerization (vs. chain propagating). Computational and experimental studies show that the functional groups can be successfully incorporated in the middle of polymer chain (Scheme S1 and Figure S58-60) and the functional groups are stable under normal storage conditions (Figure S61). We also carried out the (co)polymerization of other NCA monomers such as Lys-NCA and alkynyl functionalized Glu-NCA. All polymerizations are controlled and well-defined functionalized (co)polypeptides can be facily prepared through different monomer addition modes (Table S1).

Scheme 5. Synthesis of chain-middle functionalized polypeptide via Michael reaction and organocatalytic ROP of NCA.

Figure 6. Kinetics of the ROP of Glu-NCA promoted by **2a-2g/TU-S**, conditions: [Glu-NCA]/[**2a-2g**]/[TU-S]=120/1/2, [M] = 0.19 M, 25°C, CH₂Cl₂, the automatic sampling interval of *in situ* FT-IR is 10 seconds (The k_{app} values were obtained from the linear fits of the plots. The individual plots of $\ln([NCA]_0/[NCA]_t)$ vs. time and corresponding linear fits see Figures S51-S57).

Conclusion

In summary, by utilizing the highly efficient Michael reactions between acrylates and secondary aminoalcohols, we successfully synthesized a new family of functionalized aminoalcohols. In cooperation with thiourea organocatalyst, all resultant aminoalcohols can function as initiators for ROP of NCA, leading to well-defined, site-specific functionalized polypeptides bearing functional groups at the chain end or in the middle. The whole synthetic process is metal-free, highly active and selective at room temperature, which provides facile access to clean, functionalized polypeptides for researchers who focus on applications of polypeptide-based materials but have backgrounds other than polymer synthesis. Specially, due to the high tolerance against functional groups of the polymerization, the present method would be a potential "tool" to incorporate controlled polypeptides into natural biomolecules and inorganic molecules for bioconjugation or organic/inorganic hybridization. Further studies will be carried out to extend this strategy to other kind of NCA monomers and the synthesis of hybrid polypeptides.

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RESEARCH ARTICLE

A new synthetic methodology of site-specific functionalized polypeptides was developed. The whole synthetic process is metal-free, highly active and selective at room temperature.

Wei Zhao,* Yanfeng Lv, Ji Li,* Zihao Feng, Yonghao Ni and Nikos Hadjichristidis

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A New Synthetic Methodology of Site-Specific Functionalized Polypeptides: Metal-Free, Highly Active and Selective at Room Temperature