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Cascaded Step-Growth Polymerization for Functional Polyamides with Diverse Architectures and Stimuli Responsive Characteristics

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Abstract

Thiolactone ring opening and disulfide-thiol exchange were rationally coupled to develop a cascaded step-growth polymerization methodology for preparation of degradable polyamides. A variety of functionalities can be readily incorporated on to these polyamides by pre- and post-polymerization reactions. The polymers can be degraded into small molecules in the presence of biologically relevant reducing agents *via* backbone degradation, the kinetics of which is tunable. In addition to the redox-based chemical stimulus, the polyamides are also sensitive to light and enzymes, thus exhibiting concurrent sensitivity to chemical, physical, and biological stimuli.

Graphical Abstract

Diversity in chemical functionalities often form the basis for functional polymers with unique physiochemical properties, which have applications in a wide-range of fields from

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Author Contributions

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materials science to biology.¹ However, preparation of sophisticated polymers is often challenging due to the lack of functional group tolerance under many polymerization conditions.² The capability to incorporate a range of functionalities with control over polymer architecture should have a profound impact on expanding the inventory of functional polymers. On the other hand, there is also a need to temporally and spatially manipulate properties of interest in polymer materials.³ A variety of polymers that can sense and morph in response to a single change in the environment have been designed and utilized for this purpose.⁴ Fine control over the combination of selectivity and sensitivity is preferred in some of the more complex applications.⁵ Polymers that are capable of responding to multiple environmental changes provide a promising opportunity to rationally manipulate the performance of the resultant materials, but are much less explored due to the associated synthetic demands.⁶ In this work, we aim to simultaneously address these issues by developing a versatile cascade polymerization method for the preparation of functional polyamides that are able to simultaneously respond to physical, chemical and biological stimulus.

As shown in Scheme 1, the step growth polymerization is achieved through a combination of reaction cascades: an aminethiolactone ring opening reaction and a thiol exchange reaction. The simplicity of the reaction conditions, under which this polymerization occurs, allows for the incorporation of a variety of functional groups by employing a conveniently functionalizable thiolactone as the co-monomer. We demonstrate that this polymerization method is robust for preparing polymers with (*i*) diverse functionalities by pre- or post-functionalization; (*ii*) variations in polymeric architectures; and (*iii*) triple responsiveness to physical, chemical and biological stimuli.

Thiolactones are well-known as latent thiol reagents that undergo a ring opening reaction with nucleophiles such as amines and alcohols, via an addition-elimination mechanism to unmask the thiol group that can subsequently react with a thiol scavenger.⁷ Here, we take advantage of the fidelity of a thiol exchange reaction that has been utilized to synthesize polydisulfides⁸ and coupling this with the thiolactone ring opening to prepare disulfide containing polyamides. As shown in Scheme 1, the proposed step growth polymerization is composed of two cascade reactions, *viz.* the ring opening of functionalized homocysteine thiolactone and thiol exchange reaction. The amino group in pyridinyl disulfide ethylamine (*PDS-NH₂*), serving as a nucleophile opens thiolactone ring and generates a thiol (*PDS-M-SH*) that is further captured in situ by another pyridinyl disulfide ethylamine via thiol exchange reaction. As shown in path 1, polymer can be achieved after repeating ring opening and thiol exchange cascades. Alternatively, polymer chains can also grow through path 2 where *PDS* and thiol terminated *PDS-M-SH* undergoes thiol exchange among themselves. The nucleophilic addition forms a side-chain amide, while the disulfide bond can be embedded into amide backbone as a consequence of the thiol exchange. Diverse functional groups can be employed into polymer side chain by using modified homocysteine thiolactone which is readily accessible by amidation.

Eight monomers (**M1-M8**) with hydrophilic, hydrophobic, fluorophilic, aromatic and organometallic functional groups have been synthesized and subjected to polymerization (Figure 1). To understand the polymerization process, thiolactone ring opening and thiol

exchange reaction were simultaneously followed through NMR by monitoring disappearance of thiolactone and generation of pyridinethione when **M1** was subjected to polymerization. Molecular weight of polymer at corresponding polymerization time was also measured by GPC. As shown in Figure 2, thiolactone ring opening and thiol exchange have similar reaction kinetics during the whole polymerization. At the beginning of polymerization, around 60% of both thiolactone and pyridinyl disulfide were consumed within 15 minutes, while only oligomers were generated. Significant increase on polymer molecular weight was observed at higher monomer conversion. The relationship between monomer conversion and polymer molecular weight shown in Figure S16 suggests that the polymerization occurs through a step-growth mechanism. It has to be noted that monomer conversion and polymer molecular weight dose not significantly increase along with prolonged polymerization time at later stages. This is likely due to interpolymer thiol exchange among polymer chains or even formation of cyclic polymer. Further analysis of ¹H-NMR spectrum of **P1** indicates that the polymer is indeed composed of both the thiolactone monomer and the aminoethyl disulfide moiety (Figure S1). Additionally, the aromatic NMR peaks at 7.25, 7.80 and 8.42 ppm correspond to the pyridinyl protons and they integrate to a much smaller percentage. This indicates that the polymer is indeed terminated by the pyridinyl disulfide group. To further confirm presence of α-terminal and ω-terminal functional groups, end group was further spectroscopically analyzed by UV-vis and fluorescence. Pyridinyl disulfide was characterized by measuring absorption of pyridinethione released after treating polymer with excess reducing agent, while amine group was analyzed by fluorogenic assay where amine terminal reacts with fluorescamine to generate a fluorescent product. In order to quantitatively correlate the degree of functionality on α- and ω-termini, absorption matching fluorescence was measured. Briefly, pyridinethione absorption of both PDS-NH₂ and polymer solution treated with excess reducing agent were respectively recorded and matched to ensure that both solutions contain the same quantity of pyridine disulfide group. Then fluorogenic assay of two absorption-matched solutions of PDS-NH₂ and polymer was carried out to measure the fluorescence intensity. In such a method, the ratio of amine to pyridine disulfide can be simply evaluated by comparing fluorescence intensity between polymer and PDS, because amine to pyridine disulfide ratio in PDS-NH₂ is known to be 1. As shown in Figure S17, the ratio of pyridine disulfide and amine terminal in polymer is almost the same with that in *PDS-NH₂* and close to 1. However, we are still not able to exclude the presence of cyclic polymer since its presence has no effect on end group ratio. Homopolymerization of other functionalized monomers with pyridinyl disulfide ethylamine were also investigated to map the functional group tolerance of the cascade polymerization. As shown in Table S1, all of the investigated monomers were found to be polymerizable, except the monomer, **M8**. It is interesting that **M8** is based on a simple pyrene moiety and is therefore quite featureless for functional group interference in the polymerization. We attribute this observation to the fact that oligomers precipitated out from solution shortly after the polymerization was initiated due to the poor solubility of the oligomers, preventing further chain growth. All of above suggests the polymerization method has good tolerance for a variety of functional groups.

Next, copolymerization of two different homocysteine thiolactone monomers was also investigated to further increase the repertoire of this type of polymers. Three different

monomers, **M4**, **M6** and **M8** were selected to copolymerize with **M5**. Statistical copolymers **CP1** and **CP2** were obtained from the copolymerization of **M5** with **M4** and **M6**, respectively. The calculated ratio of the two monomers from NMR is 1:1 which corresponds to the feed ratio of the monomers, indicating similar reactivity of the two monomers during copolymerization (Figure S8, S9). In contrast, only oligomerization was observed when copolymerization of **M8** with **M2** was attempted.

Based on the proposed cascaded step growth polymerization mechanism, the α -terminal and ω -terminal on the polymer chain are pyridinyl disulfide and amino groups, respectively. Hence, the polymer itself can serve as a macromonomer which is expected to be polymerizable with second thiolactone monomer. To test the hypothesis, the chain extension was carried out by copolymerizing **CP4** with **M1**. If chain extension occurs, 2-pyridinethione which is the by-product of thiol exchange will be released from the polymer chain. So, the chain extension can be followed by measuring the release of pyridinethione, which has characteristic UV absorbance.⁹ To reduce possible cyclic form of **CP4** polymer which does not have terminal groups, **CP4** with low molecular weight was prepared at relatively low conversion and used for this study. As shown in Figure 3A, we do observe a characteristic UV-visible absorption peak at ~365 nm, suggesting the occurrence of chain extension. Additionally, the molecular weights of polymers before and after the reaction were compared to further confirm chain extension. The molecular weight was indeed found to increase from 3.1 kDa to 10.0 kDa after chain extension, which indicates that the chain extension has occurred twice (Figure 3B). The incorporation of **M1** into the final polymer was supported by the observation of signal at 1.35 ppm, which is attributed to protons from Boc (Figure 3C). The existence of ¹H NMR signal from pyridine after chain extension, but in smaller ratio, further suggests that the same polymerization mechanism operates in chain extension (Figure S10). Note that the chain extension polymerization, obtained through this method, offers the possibility of functional group incorporation at regular intervals with control.

To further expand the repertoire of functionality that can be introduced in these polymers, post-functionalization was explored. we were interested in making water soluble polymers that expands the potentially utility of this method and the associated polymers in biological applications. Functional polymers with different interfacial properties, *viz.*, cationic, anionic and zwitterionic polymers were prepared from homopolymers (**P1** and **P2**) and copolymer (**CP1**) respectively (Figure 4). Briefly, cationic polymer, **P10** bearing primary amino groups was readily obtained by the deprotection of the Boc group from **P1**, while quaternary amine containing cationic polymer **P11** was achieved by methylating the dimethylamino group in **P2** using methyl iodide (Figure S11, S12). The dimethylamino moiety in **P2** was also utilized to react with 1,3-propane-sultone to convert **P2** to the corresponding zwitterionic polymer **P12** through a ring opening reaction (Figure S13). Similarly, treatment of **CP1** with trifluoroacetic acid affords carboxylate containing anionic amphiphilic copolymer, **CP5** (Figure S14). Thus, side chain modifications provide us a convenient access to polymers with different charges which could be useful for controlling their surface potentials.

The presence of disulfide functionalities in the backbone of these polymers endows them with the potential to be degraded under reducing conditions.¹⁰ To test this rationale, redox

sensitivity of obtained polyamide, **CP1**, was investigated by dithiothreitol (DTT) incubation which is expected to reduce disulfide bonds and degrade the polymer backbone generating small molecules. NMR analysis clearly shows the degradation of polyamide, which was further confirmed by significant molecular weight decrease in GPC (Figure S18, S19). The kinetics of DTT-mediated degradation can be followed by NMR, using the formation of degradation byproducts (Figure S20–22). The percentage of cleaved disulfide can be measured by the ratio of integration of the peak at 3.2 ppm to that of peak at 4.3 ppm. As anticipated, the extent of polymer degradation and its kinetics were found to be dependent on the concentration of DTT (Figure 5A). Similar DTT-induced degradation was also observed in other polymer (Figure S23).

In addition, it has been reported that disulfide bond can be dynamic under the UV irradiation.¹¹ Utilizing this feature, we were able to degrade the polymer, **P4** for example, by exposing them to UV light. After light exposure, a significant decrease in molecular weight was observed, as discerned from GPC (Figure 5B & Figure S24). Unlike redox-mediated degradation in which polymers are completely degraded to small molecules, light-induced degradation is more likely a result of scrambling the disulfide bonds.

The polymers are composed of multiple amide bonds which also offers the possibility for enzymatic degradation. To demonstrate enzymatic responsiveness of the polymer, nanoparticles self-assembled from amphiphilic random copolymer **CP5** was treated with three different protease enzymes, namely, trypsin (TryP), chymotrypsin (ChyT), and proteinase K (PtK). The polymer was observed to form spherical particles with a size of ~200 nm range in aqueous solution by DLS and TEM (Figure S25, S26). The degradation of polymer nanoparticles was followed by monitoring the newly generated amino groups by fluorescamine assay. As shown in Figure 5C, the polymer nanoparticles are more susceptible to chymotrypsin and proteinase K, the substrates of which are hydrophobic, while the cleavage of polymer by hydrophilic-substrate-favoring trypsin is less efficient. Overall, the polymer is degradable by the enzymes from distinct susceptibility of polymer nanoparticles to different enzymes.

In summary, a cascade polymerization methodology based on thiolactone ring opening and thiol-disulfide exchange reactions has been utilized to synthesize a variety of polyamides. The method is feasible for the polymerization of a broad spectrum of monomers to afford homopolymers, random copolymers. The repertoire of the polyamides can be readily expanded to achieve functional polymers by post-functionalization. Tunable degradation of polymers can be induced by three different external stimuli, namely, redox (chemical), light (physical), and enzyme (biological). This capability of incorporating triplestimuli responsiveness in a single system provides an opportunity to finely control the performance of functional polymers, which will have significant impact on developing “smart” functional materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A: Monomers

B: Homopolymerization

C: Copolymerization

FIGURE 1:

A) Monomer structure variations; B) Synthesis of homo- and C) copolymers by cascade step growth polymerization.

FIGURE 2. Thiolactone ring opening and thiol exchange cascaded step growth polymerization of **M1** with **1**.

FIGURE 3.

A) Chain extension; B) 2-Pyridinethione release captured by absorption spectrum; C) GPC profile of polymers; D) NMR spectrum of polymers.

FIGURE 4:
Water soluble polymers prepared by post-functionalization.

FIGURE 5. Stimuli responsive degradation of polyamides. A) DTT concentration dependent disulfide cleavage; B) UV induced degradation followed by GPC; C) Enzymatic cleavage monitored by fluorescamine assay.

SCHEME 1:

Step growth polymerization comprised of thiolactone ring opening and thiol exchange cascades.