DOI: 10.1021/ma9025916

**Macromolecules**
COMMUNICATION TO THE EDITOR

# Novel Strategy for ROP of NCAs Using Thiols As Initiators: Synthesis of Diblock Copolymers Based on Polypeptides

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*Received November 27, 2009*
*Revised Manuscript Received February 17, 2010*

Block copolypeptides have recently received great attention because of their tunable chemical structures leading to possible supramolecular architectures.[1] For instance, they have been described as promising materials in applications such as biosensors,[2] tissue engineering, or selective drug delivery.[3] Usually, block copolypeptides can be obtained by two different ways: (1) the ring-opening polymerization (ROP) of *N*-carboxyanhydride (NCA) initiated by an amino-terminated polymer chain or by a transition-metal-based catalyst and (2) solid- or solution-phase peptide synthesis and subsequent coupling to a carboxyl-terminated polymer. Controlled radical polymerization techniques have also been successfully combined with ROP to obtain block copolymers containing peptide blocks.[4] Few researches were focused on dual stimuli-responsive block copolymers consisting of pH-sensitive polypeptide and thermosensitive polymer chains.[5] For example, Chang[6] succeeded in using a heterofunctional amide linkage initiator to synthesize poly(*N*-isopropylacrylamide)-*b*-polylysine by combining atom transfer radical polymerization and amine-hydrochloride-mediated ROP of lysine-based NCA. The synthesis of double hydrophilic block copolypeptides (DHBCs), associating a thermoresponsive poly(*N*-isopropylacrylamide) block and pH responsive poly (L-glutamic acid) block, was also reported by Zhang et al.[7] These authors initiated the ROP of a mixture of $\gamma$-benzyl L-glutamate- and L-lysine-based NCAs from an amino-terminated poly(*N*-isopropylacrylamide) previously prepared by RAFT polymerization.

*N*,*N*-Diethylacrylamide (DEAm), less toxic than *N*-isopropylacrylamide, is an interesting monomer because the resulting poly(*N*,*N*-diethylacrylamide) is thermoresponsive with a lower critical solution temperature (LCST) close to the one of PNIPAM, in the range of 25−35 °C.[8] To our knowledge, the synthesis and the characterization of thermoresponsive block copolymers comprising a PDEAm segment and a pH-sensitive polypeptidic segment have not been reported in the literature. We describe here the preparation of such block copolymers through a new strategy, associating ROP of NCA and RAFT polymerization of DEAm. The novelty of our approach was based on the use of a thiol-terminated[9] macroinitiator obtained via RAFT polymerization to initiate ROP of $\gamma$-benzyl-L-glutamate NCA (BLG NCA) or trifluoroacetyl-lysine NCA (TFA-Lys NCA). To date, such strategy has never been applied to combine RAFT and ROP polymerization. Because RAFT was employed for the polymerization of numerous monomers, we assume that this reactional pathway opens the way to the synthesis of many polypeptide-based block copolymers.

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Kricheldorf[10] and Deming[1,11] described the use of either amino-containing reagents or organometallic transition-metal-based complexes as initiators for ROP of *N*-carboxy anhydrides. NCA polymerization was also initiated using different nucleophiles such as alcohols or water. In this contribution, we proposed to design diblock copolypeptides in a two-step process including RAFT and ROP polymerizations where the polypeptide block was obtained from a thiol-initiated polymerization. The feasibility of the ROP of NCA by a thiol was first studied. BLG NCA was polymerized at 0 °C during 120 h in DMF using 2-(trimethylsilyl)-ethanethiol as initiator. The polymer was recovered by precipitation in a large excess of water. Poly($\gamma$-benzyl-L-glutamate) (PBLG) was characterized by $^1$H NMR spectroscopy. (See the Supporting Information.) Among all signals, the characteristic $-Si(-C\underline{H}_3)_3$ protons of the 2-(trimethylsilyl)-ethanethiol initiator were observed at 0 ppm and confirmed the incorporation of the initiator in the macromolecular chain. We concluded that the ROP of BLG NCA was achieved by this thiol. Relative integration of this peak compared with the one of characteristic protons of the PBLG main chain (e.g., methylenic proton due to the benzylic group at 5.0 ppm) permitted us to calculate the molecular weights. The obtained values were close to predicted ones coming from the [monomer]/[initiator] molar ratio (Table 1, entries 1 and 2).

Polymerizations of NCA are traditionally initiated by different nucleophiles and bases, the most common being primary amines and alkoxide anions.[10,12] Because thiols cannot be considered to be strong bases, the ring-opening mechanism of NCA can be depicted as shown in Scheme 1, where the so-called amine mechanism[10,12] was proposed. This one followed a nucleophilic ring-opening chain growth process where the polymer could grow linearly with the monomer concentration if side reactions were absent. The ROP of NCA was initiated by RSH as thiol was consumed. Its incorporation into the chain end of PBLG was demonstrated by $^1$H NMR analysis. (See the Supporting Information.) The polymerization was not considered to be a living process because the polydispersity values were $> 1.8$, even when polymerizations were achieved at 0 °C.[13] However, these results proved the feasibility of ROP of NCA initiated by thiols.

In a second step, the synthesis of diblock copolymers using thiol-terminated chains was investigated. Usually, initiation with aliphatic $NH_2$ groups is the most described method for the preparation of complex architectures such as block copolymers, dendritic structures, and star-shaped polymers and for grafting of polypeptides onto organic or inorganic solid surfaces. In all of these cases, preformed organic or inorganic polymers bearing two or more $NH_2$ groups were used as macroinitiators. As previously mentioned, Zhang[7] synthesized thermo- and pH-responsive double hydrophilic diblock copolypeptides using a new class of amino-functionalized RAFT agents. The functionalized trithio RAFT agents were prepared at low temperature to avoid any attack of the trithio group by the amino group occurring even at room temperature and leading to numerous byproduct.[14] This pathway was very difficult to achieve and needed drastic experimental conditions. In our case, a combination of RAFT and ROP polymerization was carried out using a thiol-terminated poly(*N*,*N*-diethylacrymide) (PDEAm-SH) as macroinitiator for ROP of BLG NCA and TFA-Lys NCA. DEAm was first homopolymerized by RAFT polymerization at 80 °C with *tert*-butyl dithiobenzoate as chain transfer agent and 2,2′-azobis(2-methylpropionitrile) (AIBN) as initiator with a molar ratio of

 Published on Web 03/01/2010 © 2010 American Chemical Society

**Table 1. Characteristics of the Homopolymers Synthesized by ROP Initiated by Thiols and of the Block Copolymers Prepared by ROP with Thiol-Terminated Macroinitiator**

| entry | monomer | initiator | $T$ (°C) | time (h) | ratio [M]/[I][a] | ratio [M]/[I][b] | solvent |
|---|---|---|---|---|---|---|---|
| 1 | BLG NCA | 2-(trimethylsilyl)-ethanethiol | 0 | 120 | 40 | 46 | DMF |
| 2 | BLG NCA | 2-(trimethylsilyl)-ethanethiol | 0 | 120 | 20 | 25 | DMF |
| 3 | BLG NCA | PDEAm-SH | 0 | 120 | 40 | 293 | DMF |
| 4 | TFA-Lys NCA | PDEAm-SH | 0 | 120 | 40 | 58 | DMF |

[a] Calculated molar ratio. [b] Determined by $^1$H NMR

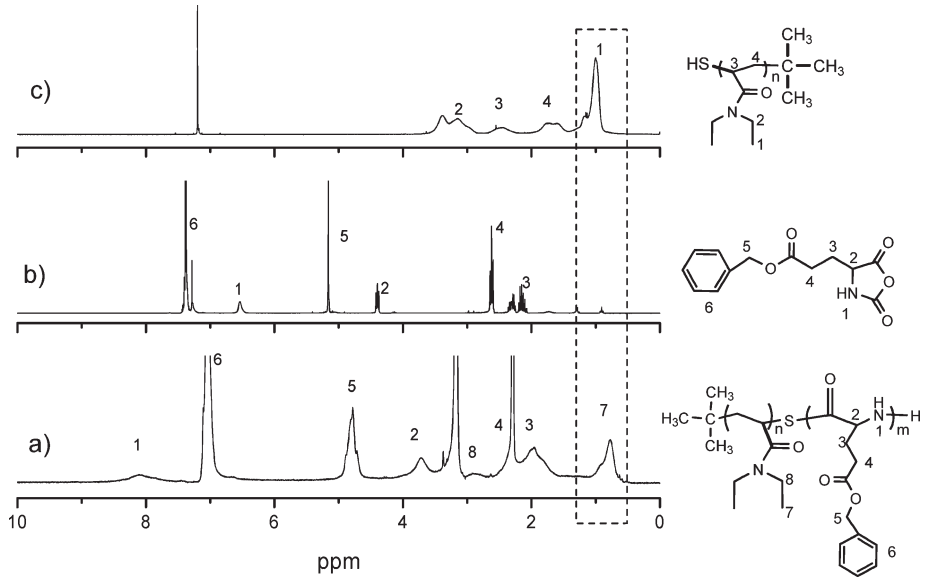
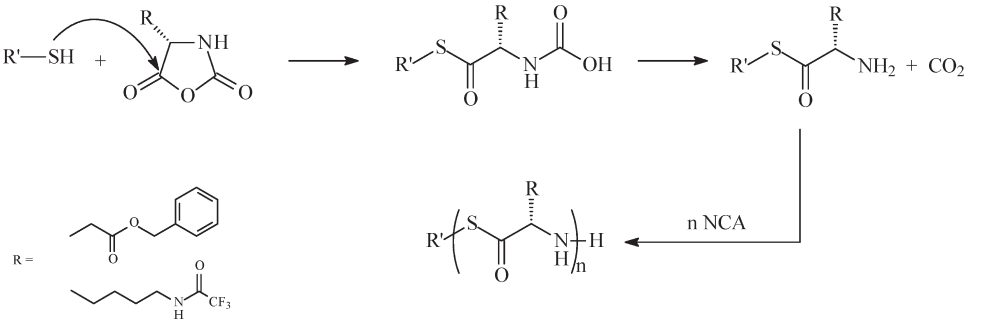



**Figure 1.** $^1$H NMR spectra of (a) the PBLG-*b*-PDEAm obtained with theoretical ratio [M]/[I] = 40:1, (b) monomer $\gamma$-benzyl-L-glutamate *N*-carboxyanhydride (BLG NCA), and (c) macroinitiator PDEAm-SH.

**Scheme 1. Proposed Mechanism for the Ring-Opening Polymerization of NCAs Initiated by Thiols**




[DEAm]/[CTA]/[AIBN] = 672:6:1 and a targeted degree of polymerization of 112 ($M_n \approx 10\,000$ g/mol).[15] The polymerization was stopped at ~70% conversion to minimize termination reactions and preserve the dithioester end groups. The resulting PDEAm was isolated by precipitation and analyzed by $^1$H NMR spectroscopy and size exclusion chromatography ($M_n$ = 8400; PDI = 1.05). Then, the dithioester end groups of the PDEAm chains were cleaved by aminolysis[14d,16–19] by reaction with the dimethylphenylphosphine to yield the PDEAm-SH without disulfide formation. The total cleavage was checked by $^1$H NMR spectroscopy, where no signals in the aromatic region associated with the phenyl end group of the starting homopolymer was observed, confirming the successful end-group cleavage. (See the Supporting Information.) The UV−vis spectroscopy showed the complete disappearance of the absorbance at 310 nm associated with the C=S bond.[20] Furthermore, macromolecular disulfides that were produced during reaction were observed on SEC trace (shoulder on curve for $t$ = 2 h) but did not remain at the end of reaction.

At last, the thiol-terminated PDEAm was used as macroinitiator for the ROP of BLG NCA and TFA-Lys NCA (Table 1, entries 3 and 4). The PBLG-*b*-PDEAm samples were characterized by $^1$H NMR spectroscopy. As illustrated in Figure 1 showing $^1$H NMR spectra of PDEAm-SH, BLG NCA, and of the PBLG-*b*-PDEAm copolymer, all expected peaks were observed, confirming the ROP of BLG NCA by the PDEAm-SH. In particular, characteristic protons arising from the macroinitiator PDEAm-SH were clearly identified. For instance, the methyl protons from the PDEAm block were observed at 0.9 to 1.0 ppm. Relative integration of this peak compared with the methylenic protons of benzyl group in the PBLG block at 4.9 to 5.0 ppm allowed us to determine a degree of polymerization close to 293. SEC traces also showed a clear shift of the peak corresponding to the functional PDEAm block toward the higher molecular weights region after ROP of TFA-L-lysine with no evidence of unreacted macroinitiator (Figure 2). We also observed a shoulder in the higher molecular weight region due to some uncontrolled polymerization. The latter could be due to the homopolymerization

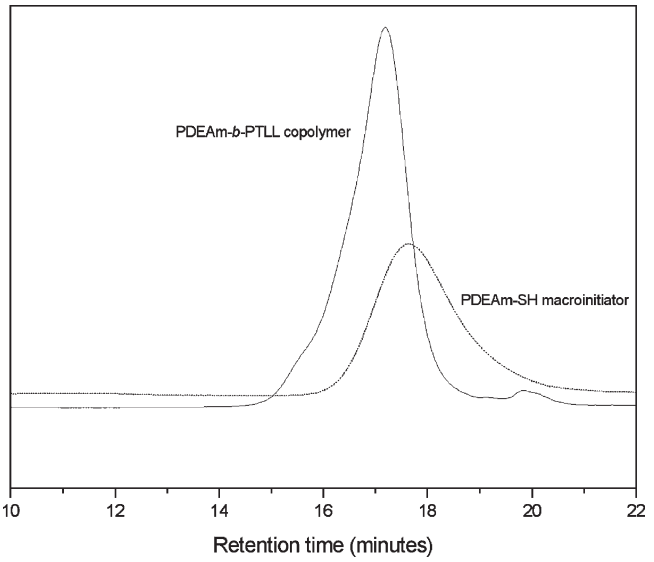



**Figure 2.** Size exclusion chromatography traces of PTLL-*b*-PDEAm (Table 1, entry 4) and PDEAm-SH macroinitiator. Eluent: DMF; PS standards.

reaction from impurities found in the DMF used as solvent. Finally, P(TFA-L-lysine) block was deprotected using ammoniac aqueous solution to afford PDEAm-*b*-P(L-lysine), sensitive to both temperature and pH. UV−vis experiments permitted us to prove that the LSCT was dependent on the pH value. (See the Supporting Information.)

In conclusion, a new approach for the synthesis of polypeptide-based diblock copolymers has been described combining RAFT and ROP polymerizations. We proposed a novel synthetic pathway to lead to peptidic block copolymers using thiol-terminated macroinitiator instead of usual $NH_2$ terminated one for the ROP of NCAs. The thiol-terminated PDEAm macroinitiator was obtained via the selective aminolysis of a dithioester moiety resulting in the RAFT polymerization of DEAm. The ROP of NCA initiated by a thiol group seemed to follow a "normal amine route", as shown during the homopolymerization of BLG NCA and TFA-Lys NCA. A further study will examine the influence of experimental parameters on kinetics (solubility and chemical structure of the growing peptide chain, termination reactions, etc.) to achieve a living ROP.

**Supporting Information Available:** Experimental procedures and characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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