Tuning LCST with thiol-responsiveness of thermoresponsive copolymers containing pendant disulfides

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A new approach that centers on modulating hydrophobic/hydrophilic balance by conversion of pendant disulfides to thiols, and further to sulfides enables facile tuning of the thermoresponsive properties of thiol-responsive copolymers.

Stimuli-responsive polymers undergoing phase transition in response to external stimuli hold a great promise for various applications.[[1-2](#_ENREF_1)] In particular, thermoresponsive polymers possessing lower critical solution temperature (LCST) behaviour are hydrophilic and swell at low temperature, facilitating encapsulation; above LCST, however, they are hydrophobic and shrink, enhancing release.[[3-4](#_ENREF_3)] Poly(N-substituted acrylamide) is representative of the class of thermoresponsive polymers; among these, poly(N-isopropylacrylamide) (PNIPAM) is the most studied polymer due to its near body temperature LCST driven response at 32 °C.[[5-6](#_ENREF_5)] However, PNIPAM-based copolymers have been known to be cytotoxic, suggesting that their use for biomedical applications may be limited.[[7](#_ENREF_7)] Polymethacrylates with pendant ethylene oxide (EO) units (i.e. poly(oligo(ethylene oxide) monomethyl ether methacrylate, POEOMA) present a promising alternative.[[8](#_ENREF_8)] POEOMA-based polymers are analogs of poly(ethylene oxide), a readily used FDA-approved material due to its biocompatibility and low toxicity.[[9-10](#_ENREF_9)] More importantly, they exhibit tunable LCST between 20 and 90 °C by varing the composition of POEOMA with different number of EO units in side chains.[[11](#_ENREF_11)] These unique properties promote the use of POEOMA-based materials for various biological and biomedical applications.[[12](#_ENREF_12)]



**Scheme 1**. A new approach to tune LCST of thiol-responsive thermoresponsive POEOMA copolymers by modulating pendant hydrophobic/hydrophilic balance with disulfide-thiol-sulfide chemistry.

Stimuli-responsive degradation (SRD) is a promising property that enables enhancing the release of encapsulated guest molecules as well as tuning the morphologies of self-assembled nanostructures.[[13-14](#_ENREF_13)] Particularly, disulfide linkages can be incorporated into designer nanomaterials, and then cleaved to the corresponding thiols in a reducing environment or through a disulfide-thiol exchange.[[15](#_ENREF_15)] In biological systems, glutathione (GSH) is found at millimolar concentrations in normal cells, and at further elevated levels in cancer cells.[[16](#_ENREF_16)] The thiol-disulfide degradation property offers an advantageous SRD platform in constructing thiol-responsive degradable nanomaterials desirable for various biomedical applications; these include micelles,[[17-19](#_ENREF_17)] nanocapsules,[[20](#_ENREF_20)] nanogels,[[21-22](#_ENREF_21)] hydrogels,[[23-24](#_ENREF_23)] and bioconjugates.[[25-27](#_ENREF_25)] Recently, this property has also been utilized to adjust thermoresponsive properties of thiol-responsive polydisulfides of multisegmented POEOMA[[28](#_ENREF_28)] and PNIPAM[[29](#_ENREF_29)] upon redox-responsive cleavage of main chain disulfide linkages.

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Herein, we report a new method to finely tune thermoresponsive properties of thiol-responsive POEOMA copolymers containing pendant disulfide linkages by modulating their hydrophobic/hydrophilic balance with disulfide-thiol-sulfide chemistry. As shown in Scheme 1, an introduction of a hydrophobic polymethacrylate functionalized with pendant disulfide linkages (HMssEt) increases the hydrophobicity of POEOMA copolymers, decreasing the LCST. Different amounts of HMssEt are copolymerized with a mixture of OEOMA300 (MW = 300 g/mol with pendant EO units = 5) and di(ethylene glycol) monomethyl ether methacrylate (DEGMA, MW = 189 g/mol and pendant EO units = 2). Controlled radical polymerization (CRP)[[30](#_ENREF_30)] is utilized for the synthesis of well-controlled P(OEOMA-co-HMssEt) copolymers with different densities of pendant disulfide linkages. Next, cleavage of the pendant disulfide linkages to the corresponding thiols in response to external reducing agents increases the hydrophilicity and the LCST. Furthermore, the resulting pendant thiols are converted to sulfide linkages through thiol-ene Michael addition with enones (i.e. acrylates),[[31-32](#_ENREF_31)] decreasing hydrophilicity, and thus decreasing the LCST. Our approach is promising in that the further modification of pendant thiols through thiol-ene Michael addition or thiol-disulfide exchange reaction will allow for the incorporation of additional reactive moieties or the conjugation of designed therapeutics for biological applications.

A new methacrylate functionalized with a pendant disulfide linkage, HMssEt, was synthesized (Scheme S1), and characterized using 1H-, 13C-NMR, and high resolution mass spectroscopies, as described in our previous publication.[[33](#_ENREF_33)] Scheme S2 (supporting information) illustrates the synthesis of well-defined P(OEOMA-co-HMssEt) copolymers (ssCPs) using atom transfer radical polymerization (ATRP),[[34](#_ENREF_34)] a successful CRP method. A series of ATRP was catalyzed with CuBr/PMDETA (*N*,*N*,*N*′,*N*′′,*N*′′-pentamethyldiethylenetriamine) complexes in the presence ethyl α-bromoisobutyrate initiator in acetone at 47 °C. The amounts of HMssEt were varied at 0 – 10 mol% in monomer mixtures. Table 1 summarizes the characteristics of ssCP copolymers.



**Fig 1.** 1H-NMR (a) and temperature dependence of normalized light scattering intensity by DLS (b) of ssCP-6 with pendant disulfide linkages, shCP-6 with pendant thiols formed by cleavage of pendant disulfides in response to DTT, and sCP-6 with pendant sulfides converted by thiol-ene reactions with tBA.

**Table 1.** Characteristics of ssCP copolymers prepared by ATRP.a

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ssCP | HMssEt (mol%) | Convb | Mnc (g/mol) | Mw/Mnc | LCSTd (°C) |
| 0 | 0 | 0.29 | 10,200 | 1.28 | 48.5 |
| 2 | 2 | 0.37 | 11,300 | 1.28 | 42.3 |
| 2H | 2 | 0.64 | 17,400 | 1.31 | 38.3 |
| 4 | 4 | 0.30 | 11,700 | 1.24 | 33.6 |
| 6 | 6 | 0.29 | 11,300 | 1.27 | 29.2 |
| 10 | 10 | 0.31 | 12,400 | 1.22 | - |

a. Conditions: [monomers]0/[EBiB]0/[CuBr/PMDETA]0 = 120/1/0.5, [OEOMA]0/[DEGMA]0 = 0.38/1, monomers/anisole = 1/1 wt/wt.

b. Determined by 1H-NMR

c. Determined by GPC using DMF as an eluent with poly(methyl methacrylate) standards.

d. Determined by DLS

For kinetic studies, aliquots were taken to determine monomer conversion using 1H-NMR and molecular weight and molecular weight distribution using gel permeation chromatography (GPC). As seen in Fig. S1a, all polymerizations had first-order kinetics, suggesting constant concentration of active centers. Monomer conversion reached over 60% in 4 hrs, suggesting no significant effect of the amount of HMssEt on the rate of polymerization. Molecular weight increased linearly with conversion and molecular weight distribution remained relatively narrow with Mw/Mn < 1.4 (Fig S1b). Furthermore, GPC traces evolved to the high molecular weight region over the course of the polymerization (Fig S2). These results suggest that ATRP of mixtures of OEOMA and HMssEt proceeded in a living fashion.

The purified ssCPs are thermoresponsive and dynamic light scattering (DLS) technique was used to examine thermoresponsive behavior of these ssCPs with different amounts of HMssEt units. Light scattering (LS) intensity of the aqueous copolymer solutions at 0.1 mg/mL (0.1 wt%) was measured with temperatures varying from 20 to 85 °C at increments of 1 °C. The effect of the amount of hydrophobic HMssEt units in ssCPs was first examined. Note that molecular weights of five copolymers (ssCP-0, 2, 4, 6, and 10 in Table 1) ranged from Mn = 10-12 kg/mol. As seen in Table 1, LCST decreased from 48.5 °C to 29.2 °C with an increasing amount of hydrophobic HMssEt units from 0 to 6 mol%. Such significant decrease in LCST is attributed to increasing hydrophobicity of ssCPs. Note that ssCP-10 with 10 mol% HMssEt formed aggregates in aqueous solutions. The effect of molecular weight was also examined. For copolymers with 2 mol% HMssEt, DLS results show that the LCST decreased from 42.3 to 38.3 °C when molecular weight increased from Mn = 11.3 kg/mol (ssCP-2) to 17.4 kg/mol (ssCP-2H) (Fig S3). Similar molecular weight dependent results are reported elsewhere.[[35](#_ENREF_35)]

Next, pendant disulfide linkages of ssCPs were cleaved in the presence of D,L-dithiothreitol (DTT), a well-known amphiphilic thiol, in DMF. 1H-NMR was used to monitor the cleavage of disulfide linkages. As seen in 1H-NMR spectrum of ssCP-6 as a typical example (Fig 1a, upper), the peaks at 2.9 ppm correspond to two methylene protons adjacent to disulfides (c and d) and peaks at 2.7-2.8 ppm correspond to two methylene protons adjacent to carbonyl (-C=O) groups (b and e). Upon the cleavage of disulfides to corresponding thiols (Fig 1a, middle), peaks (d and e) disappeared (due to the removal of small thiol moieties during precipitation) and peak (c) was shifted to 2.7 ppm (c′) (due to the conversion of disulfide to thiol). In addition, a new triplet at 1.5 ppm corresponding to thiol (-SH) proton (g) appeared. These results confirm the pronounced cleavage of pendant disulfide linkages of ssCPs to form the corresponding P(OEOMA-co-HMSH) copolymers having pendant thiol (-SH) groups (shCPs).

Fig 1b shows a typical example of thermal properties of ssCP-6 with 6 mol% HMssEt, showing an increase in LCST from 29.2 to 38.3 °C before and after cleavage of pendant disulfide linkages. For ssCP-4 with 4 mol% HMssEt, its LCST also increased from 33.2 to 42.3 °C after the cleavage of pendant disulfide linkages. This considerable increase in LCST by ca. 10 °C can be attributed to the cleavage of pendant disulfides to thiols, changing the hydrophilic/hydrophobic balance in the pendant chains of ssCPs. Note that no significant effect of residual DTT on thermal properties of shCPs is observed (Fig S4).

Further, the resulting hydrophilic thiols were reacted with t-butyl acrylate (tBA) in the presence of n-butyl amine. After purification to remove excess tBA, 1H-NMR (Fig 1a, lower) shows a typical peak at 1.5 ppm corresponding to nine tertiary methyl protons (h) and peaks at 2.5 ppm corresponding to two methylene protons adjacent to sulfide linkage (j), suggesting the formation of P(OEOMA-co-HMstBA) (sCP-6). The conversion of thiols to sulfide linkages was determined to be 38% (see supporting information and Fig S5). As seen in Fig 1b, the DLS results indicate the LCST decreasing from 38.2 to 35.3 °C as a result from conversion of hydrophilic thiols to hydrophobic sulfides (decreasing hydrophilicity).

In conclusion, we demonstrated a new approach to tuning thermoresponsive properties of thiol-responsive POEOMA copolymers by modulating the pendant hydrophobic/hydrophilic balance with disulfide-thiol-sulfide conversions. The LCST decreased with increasing incorporation of hydrophobic HMssEt monomer functionalized with pendant disulfide linkage. The LCST could then be increased by thiol-responsive cleavage of pendant disulfide linkages to the corresponding thiols; and further decreased by thiol-ene reactions to convert sulfides. These results suggest that the new approach will be useful in constructing novel nanomaterials requiring tunable LCST behaviour for biological and biomedical applications.

This research was supported by the NSERC Canada, Canada Research Chair (CRC) Award, and partially Advanced Technology Center program (10032218) in the Korean Ministry of Knowledge Economy. KRB thanks for NSERC Undergraduate Summer Research Award (USRA).

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