Dual Stimuli-Responsive Nanogels by Self-Assembly of Polysaccharides Lightly Grafted with Thiol-Terminated Poly(*N*-isopropylacrylamide) Chains

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Nanometer-sized hydrogels (nanogels), which exhibit properties of both nanoparticles and hydrogels, have attracted much attention as objects of study in basic science and as components of materials for cosmetics, biotechnology, and medicine.[1] They have been prepared by techniques such as microemulsion polymerization, intra- and inter- polymer chains cross-linking, and short pulse irradiation.[2] They can also be generated by self-assembly of amphiphilic polymers bearing hydrophobic substituents, which in water form physically cross-linked nanogels,[3] such as cholesteryl-modified pullulans shown to be effective nanocarriers for hydrophobic drugs and proteins.[4] The work described here concerns the preparation of nanogels by heat-induced association of polysaccharides sparingly grafted with short poly(*N*-isopropylacrylamide) (PNIPAM) chains. At room temperature, these polymers readily dissolve in water. Above a lower critical solution temperature (LCST), PNIPAM-*g*-polysaccharides form nanogels physically cross-linked by the hydrophobic nanodomains generated by dehydration of PNIPAM. The nanogel building blocks were obtained by grafting short PNIPAM chains (M_n = 800−4000 g/mol) onto pullulan (Pul) by reversible addition-fragmentation chain transfer (RAFT) polymerization of NIPAM in water controlled by sulfanylthiocarbonylsulfanyl (STS) groups covalently attached to the pullulan framework acting as chain transfer agents (Figure 1). The method offers the unique advantage of yielding polymers with STS groups linked to the end of each PNIPAM chain. Aminolysis of STS groups generates thiols,[5] which in the presence of an oxidant readily form disulfides yielding chemically cross-linked nanogels able to withstand changes in temperature. Previous routes toward PNIPAM-grafted polysaccharides, such as postpolymerization coupling of PNIPAM, cerium-catalyzed grafting of PNIPAM, or atom transfer radical polymerization (ATRP) grafting of NIPAM, do not provide this opportunity.[6] Overall, the self-assembly of PNIPAM-*g*-polysaccharides with thiol end groups (Figure 1) produces nanogels responsive to two stimuli: temperature and/or redox conditions. This adds an important parameter to be manipulated in designing the controlled release of active agents entrapped in nanogels.

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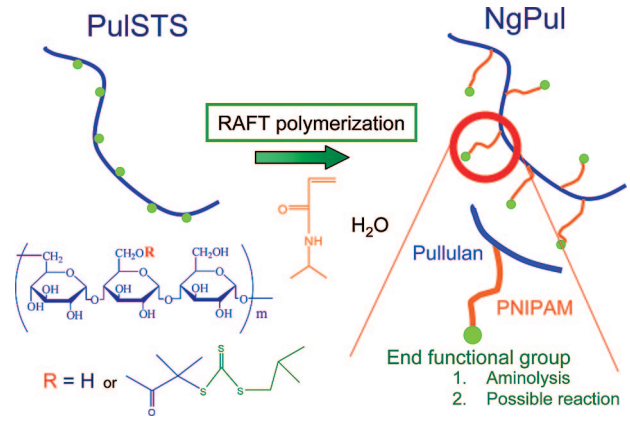

Figure 1. Schematic representation of PulSTS and the preparation of NgPuls.

Dually responsive systems are promising multi functional systems, in particular in therapeutic applications were they can act as drug carriers for intracellular delivery.[7]

The macro-chain transfer agent PulSTS was synthesized by coupling 2-(1-isobutyl)-sulfanylthiocarbonylsulfanyl-2-methyl propionyl acid chloride[8] to pullulan in the presence of 4-dimethylaminopyridine and triethylamine. The number of STS groups linked to pullulan ranged from 1.6 to 4.0 per 100 glucose units (or 10 − 25 STS per pullulan chain) depending on the initial ratio of pullulan to acid chloride (Figure S1). The polymerization of NIPAM was carried out in water at 60 °C for approximately 3 h in the presence of the water-soluble initiator 2,2′-azobis[2-(2-imidazolin-2-yl)propane] using three different macro- chain transfer agents (5.0 mg/mL of PulSTS). The molar ratio of NIPAM to STS units ranged from 10−40 and the [STS]/[Initiator] ratio was kept constant at 6/1. The monomer conversion was 70−85%, as estimated from the disappearance of signals due to NIPAM in the ^{1}H NMR spectra of the polymerization solutions (Figure S2). To ensure the fast response of the nanogels to changes in temperature, the length of the PNIPAM chain needs to be short.[9] It was adjusted by varying the NIPAM feed. Thus, with PulSTS3.3 (3.3 STS units per 100 glucose units), we prepared 4 samples, N7gPul, N13gPul, N26gPul, and N34gPul, for which the average number of NIPAM units per grafted PNIPAM chain was 7, 13, 26, and 34, respectively. The relative molar amounts of pullulan units to NIPAM units in the NgPuls were obtained from their ^{1}H NMR spectra, using the signals at 0.85−0.92 ppm due to the resonances of the methyl protons of STS group, 0.98−1.18 ppm due to the methyl protons of the NIPAM units, and the signals at 5.22−5.36 ppm ascribed to the resonances of the anomeric proton *1H* (1−4) of the pullulan glucose units (Figure S3).

Differential scanning calorimetry (DSC) revealed that aqueous solutions of all NgPuls undergo a phase transition upon heating with a transition temperature (T_{max}, taken as the maximum of the endotherm) ranging from 49.2 °C (N7gPul) to 35.3 °C (N34gPul) (Figure 2). Similar T_m values have been observed for PNIPAM oligomers, although comparisons can only be qualitative given the important effect of end groups on the phase transition of short chains.[9]

The heat-induced collapse of the PNIPAM grafted chains induced significant changes. In cold aqueous solution, NgPuls do not form stable self-assembled structures since the

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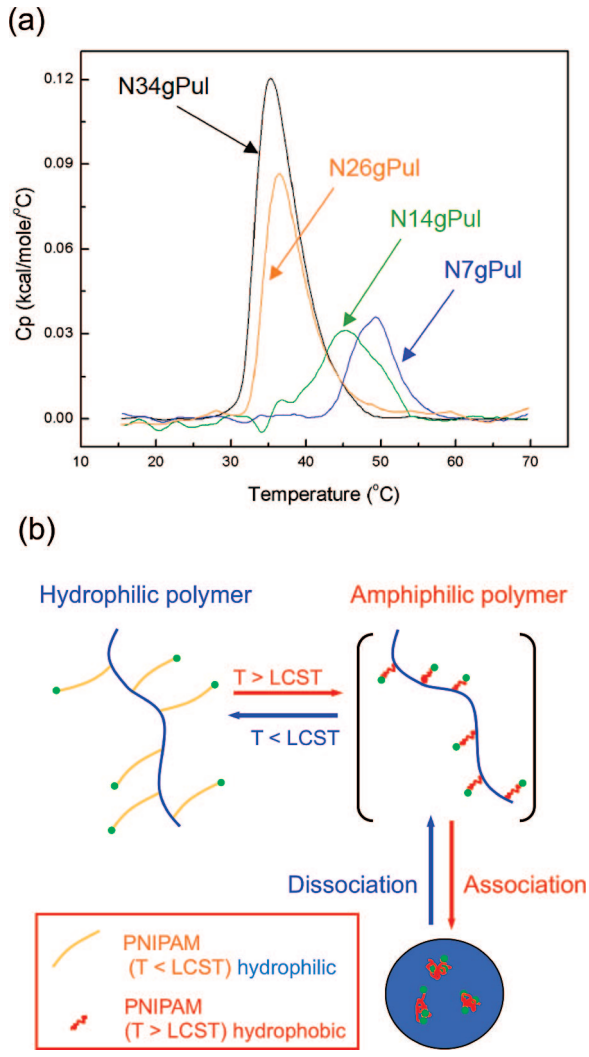

Figure 2. (a) Microcalorimetric endotherms of aqueous solutions of various NgPul samples. Polymer concentration: 3.0 g L^{-1}. Heating rate: 60 °C h^{-1}. (b) Schematic representation of thermally induced nanogels of NgPul.

Table 1. Characterization of NgPuls

abbr	T_{max} (°C)[a]	ΔH (kcal mol^{-1})[a]	R_h (nm) (PDI)[b]
N7gPul	49.2 ± 0.1	248 ± 37	56.6 (0.37)
N13gPul	45.9 ± 0.6	340 ± 45	29.9 (0.40)
N26gPul	36.2 ± 0.3	623 ± 36	23.9 (0.17)
N34gPul	35.3 ± 0.1	820 ± 34	20.2 (0.13)

[a] Determined by high sensitivity differential scanning calorimetry. [b] Determined by dynamic light scattering at 50 °C.

PNIPAM grafts are hydrophilic. Increasing the solution temperature from 25 to 50 °C triggered the formation of particles of narrow size distribution with a hydrodynamic radius (R_h) of 20−25 nm, in the case of the nanogels bearing the longest PNIPAM chains, N34gPul and N26gPul (Table 1). These nanogels are cross-linked through hydrophobic domains generated by the association of the dehydrated PNIPAM chains of PNIPAM-*graft*-pullulan. The association is fully reversible: cooling the solutions to room temperature resulted in the dissociation of nanogels. Although N13gPul and N7gPul also underwent a temperature-induced phase transition, the self-assembled structures formed in solutions heated above 50 °C, were of much broader size distribution, compared to N34gPul or N26gPul (Table 1).

The dual responsive nature of the nanogels was tested using the sample N26gPul, as depicted in Figure 3a. First, the STS-bearing N26gPul was treated with isopropylamine yielding the copolymer SH-N26gPul having thiol-terminated grafts, as confirmed by the disappearance of the UV absorbance (λ_{max} = 298 nm) characteristic of the STS group (Figure S4)[10] and a positive response to the Ellman colorimetric assay for thiols. The SH-N26gPul was purified quickly under oxygen-free conditions. A solution of the resulting polymer was brought to 50 °C and purged with air to trigger the conversion of thiols to disulfides. Conversion of the thiols to disulfides was complete after ~24 h, at which point the air flow was discontinued and the solution was brought to room temperature. Microcalorimetry scans were recorded for solutions of disulfide cross-linked N26gPul (SS-N26gPul). The T_m was 35.9 ± 0.1 °C and the ΔH was 836 ± 89 kcal/mol. The T_m value of SS-N26gPul decreased somewhat, compared to its value before aminolysis. The small difference reflects the balance of two opposite effects: (1) increased hydrophilicity, hence higher T_m, as a consequence of the removal of the isobutyl end group by aminolysis, and (2) increased molecular weight of the PNIPAM chains as a result of cross-linking via disulfide bond formation, which should lead to decrease of the transition temperature. The SS-N26gPul formed nanoparticles with a hydrodynamic radius of 86.7 nm in aqueous solutions kept at 25 °C, a temperature lower than LCST of the PNIPAM grafted chains. Thus, the particles did not disintegrate below the LCST, confirming the formation of disulfide cross-links. Reheating a solution of SS-N26gPul nanogels to 40 °C, led to a sharp decrease in the size and size distribution of the nanogels (R_h = 54.5 nm, ~ 40% in terms of hydrodynamic radius) as a result of the collapse of the PNIPAM chains (Figure 3b). As the solution was brought back to 25 °C, the SS-N26gPul nanogels regained the size they had before the heat treatment (R_h = 87.2 nm), confirming that SS-N26gPul nanogels are thermo- responsive. In water kept below the LCST, the SS-N26gPul are hydrophilic nanogels held together solely via disulfide bonds. Above the LCST, association of collapsed hydrophobic PNIPAM chains creates additional physical cross-linking points.

The resulting nano network can be relaxed either upon cooling to room temperature or upon treatment with a reducing agent. Treatment of a cold (25 °C) solution of SS-N26gPul nanogels with a reducing agent (tris(2-carboxyethyl)phosphine, TCEP) generated a suspension of nondistinctive size characteristics similar to the original sample. Under these conditions both the chemical and physical cross-linking points are destroyed, and consequently, the nanogels unravel. The reduction of disulfide bond in SS-N26gPul was also performed at 40 °C in the presence of 10 mM TCEP and the changes of nanogels size were tracked by DLS. The hydrodynamic radius of the nanogels increased to ~95 nm (at 24 h) and their size distribution remained narrow (PDI ~ 0.12). These characteristics are quite similar to those of SH-N26gPul obtained immediately upon just aminolysis of the STS groups. (The hydrodynamic radius was 102.3 nm with the PDI of 0.05 at 50 °C.)

In summary, we have prepared dual stimuli-responsive nanogels via RAFT polymerization of NIPAM from STS attached to pullulan and cross-linked the grafted chain ends through disulfide bonds generated from the STS groups after polymerization. The same approach can be taken using monomers other than NIPAM and can be extended easily to the preparation of block copolymer grafted chains. Therefore, RAFT polymerization of PulSTS enables one to create various types of stimuli responsive nanomaterials. This design strategy of functional nanogels by self-assembly of associat-

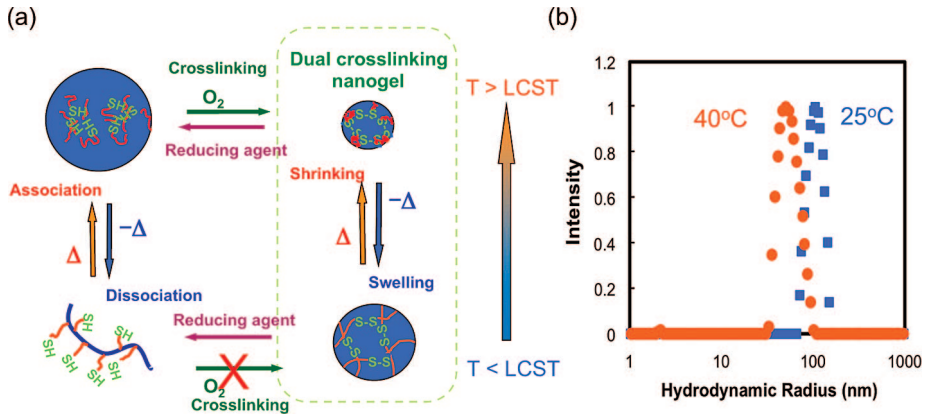

Figure 3. (a) Schematic representation of temperature and redox responsive SS-N26gPul nanogels constructed by RAFT polymerization of *N*-isopropylacrylamide onto PulSTS. (b) Hydrodynamic radius of the dual responsive SS-N26gPul nanogels in water below and above LCST.

ing and functional polymers is useful for the developments of new nanobiomaterials.

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Supporting Information Available: Text giving full experimental details, a table of RAFT polymerization data, and figures showing ^{1}H NMR of STS substituted pullulan, pseudo first-order kinetic plot for N7gPul, ^{1}H NMR of N7gPul, UV−vis spectra of N26gPul, efficiency of the cross-linking reaction, and changes of the hydrodynamic radius of N26gPul. This material is available free of charge via the Internet at http://pubs.acs.org.

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