Polymers

Thermosensitive Triterpenoid-Appended Polymers with Broad Temperature Tunability Regulated by Host–Guest Chemistry

Jie Hao,[a] Yuxia Gao,[a] Ying Li,[b,d] Qiang Yan,*[c] Jun Hu,*[b] and Yong Ju*[a]

Abstract: Thermoresponsive water-soluble polymers are of great importance since they typically show a lower critical solution temperature (LCST) in aqueous media. In this research, the LCST change in broad temperature ranges of copolymers composed of natural glycyrrhetic acid (GA)-based methacrylate and N,N'-dimethylacrylamides (DMAs) was investigated as a function of the concentration and the content of GA pendants. By complexation of GA pendants with β-cyclodextrin (β-CD), a side-chain polypeudorotaxane was obtained, which exhibited a significant increase in the LCST of copolymers. Moreover, the precisely reversible control of the LCST behavior was realized through adding a competing guest molecule, sodium 1-admantylcarboxylate. This work illustrates a simple and effective approach to endow water-soluble polymers with broad temperature tunability and helps us further understand the effect of a biocompatible host–guest complementary β-CD/GA pair on the thermoresponsive process.

Introduction

Stimuli-responsive polymers, defined as a kind of smart macromolecule, can be remarkably changed in a controlled fashion by external stimuli, such as temperature, pH, metal ions, gases, redox agents, and light.[1] Among them, thermosensitive polymers are the most widely studied since they exhibit a lower critical solution temperature (LCST) in aqueous media. Such polymers can undergo a phase transition from a soluble to an insoluble state upon heating above the LCST.[2] To adapt to the requirements of modern materials science and nanomedicine, enormous efforts have been devoted to precisely controlling the LCST of thermosensitive polymers in recent years.[3]

One efficient strategy is to introduce host–guest chemistry through combining multiple dynamic interactions and forming reversible inclusion complexes. Cyclodextrins (CDs), which can form inclusion complexes with small molecules and polymers, have been widely used as supramolecular hosts.[4] By utilizing the hydrophilic exterior and hydrophobic interior, CDs can regulate the polymeric thermosensitivity by side or end guest groups.[5] For example, Wang and co-workers showed the effect of CDs on the LCST of poly(N,N’-dimethylaminomethylmethacrylate) (PDMAMA), poly(N-n-propylmethacrylamide) (PnPMAm), and poly(N-isopropylacrylamide) (PnIPAAm) and found the micellization temperature of PnPMAm increased significantly in the presence of α-CD on account of the complex formation of α-CD with the n-propyl group.[5e] Peng’s group reported that azobenzene-containing polymers with α-CD provided tunable solubility in aqueous solution at a certain temperature owing to photoinduced complexation and decomplexation.[5f] Although these studies are highly interesting, the materials have limitations in potential biological applications. Thus, it is necessary to explore new host–guest pairs, especially composed of biocompatible molecules, to tune the thermoresponsiveness of water-soluble polymers.

Glycyrrhetic acid (GA), a natural pentacyclic triterpenoid, has abundant pharmacological activities, such as anti-inflammatory, antiviral, and anticancer effects.[6] Recent studies show GA is an ideal building block for fabricating self-assembled biocompatible materials because of its rigid skeleton, chiral centers, multiple reactive sites, and good biocompatibility.[7] In our previous work,[7e] GA was used as a novel guest molecule to bind β-CD to form a GA/β-CD inclusion complex. By incorporating GA and β-CD pendants into poly(N,N’-dimethylacrylamide), respectively, we obtained two types of copolymers, named poly(DMA-GA) and poly(DMA-CD). On account of the
natural origin of GA and β-CD, these polymers showed outstanding biocompatibility in a CellTiter-Blue cell viability assay. Additionally, a supramolecular self-healable polymeric hydrogel was prepared through physically cross-linking these two polymers induced by the host–guest interaction between GA and β-CD. Thus, β-CD/GA is expected to become a new host–guest pair in biocompatible materials. To understand how the complexation of GA with β-CD affects the thermosensitivity of poly(DMA-GA), in this paper we studied the LCST change of poly(DMA-GA) as a function of the concentration and the number of GA pendants. By adding β-CD into the aqueous solution of poly(DMA-GA), we obtained a side-chain polypseudorotaxane and explored the influence of the host–guest interaction on its thermal properties. Moreover, the precisely reversible control of the LCST behavior was realized by adding a competing guest molecule, sodium 1-admantylcarboxylate (Figure 1).

Results and Discussion

Synthesis of Poly(DMA-GA) Copolymers

Statistical copolymers poly(DMA-GA-x%) (x = 0, 1, 2, 4) bearing GA pendants with different contents were synthesized by means of a free-radical polymerization of N,N'-dimethylacrylamide (DMA) with GA-based methacrylate according to our previous work. Their molecular weights, polydispersity indexes (PDIs), and yields are summarized in the Supporting Information. The 1H NMR spectrum of the resultant poly(DMA-GA-2%) at 25 °C in D2O is shown in Figure 2A.

Thermoresponsive Behavior of Poly(DMA-GA) Copolymers

It is well known that poly(DMA) has excellent water solubility even at higher temperatures, and the copolymerization with hydrophobic monomers can endow poly(DMA) with a thermal phase-transition feature, which could be turbid at elevated temperature. Hence, we supposed that DMA copolymerized with the GA-appended monomer unit can yield thermoresponsive copolymers, as shown in the Supporting Information.

Figure 1. Chemical structure and illustration of the tunable thermoresponsive behaviors of poly(DMA-GA).

UV/Vis spectroscopy was used to record the turbidity of the copolymer solution against temperature change, as shown in Figure 3A. The results showed that the LCST behavior of poly-
(DMA-GA-x%) highly depends on the polymer composition: 1) the poly(DMA) without GA pendants has excellent water solubility and never underwent phase separation even at higher temperature; 2) the LCST of poly(DMA-GA-1%) was 79°C; this dropped to 46°C for poly(DMA-GA-2%) at the same concentration, which indicated that the higher number of hydrophobic GA pendants made the phase separation sharper and occur more easily at lower temperatures; 3) below the LCST, an obvious difference in the light transmittance was observed for poly(DMA-GA-2%) and poly(DMA-GA-4%), which means that poly(DMA-GA-4%) was so cloudy that the light absorption of the aqueous solution was too high at the initial temperature; this was mainly caused by the high molar ratio of GA monomers, thus raising the hydrophobicity of the copolymer and leading to the formation of larger polymeric aggregates initially (see the Supporting Information); 4) the LCST of the thermoresponsive polymer was able to be adjusted over a long range (around 40°C) by means of a simple and effective way to change the molar ratio of GA monomers.

The effect of copolymer concentrations on the LCST behavior was also determined by turbidity measurements. Figure 3B showed the thermal sensitivity of the aqueous solution of poly(DMA-GA-1%) under different concentrations (the molar concentration of GA units was 1.36–3.62 mmolL⁻¹). The LCST decreased from 79 to 67°C with the increase of concentration from 15 to 40 gL⁻¹, and gradually became constant at higher copolymer concentration, which was consistent with a transition from hydrated random coils to hydrophobic globules as reported by Fujishige and co-workers.[9]

The phase-transition temperature of poly(DMA-GA-1%) was quite high even at a concentration of 40 gL⁻¹, which resulted from the low number of GA pendants in the copolymer, thus making its use difficult. Therefore, the phase transition of poly(DMA-GA-2%) with a higher molar ratio of GA was studied (the molar concentration of the GA units was 0.88–2.93 mmolL⁻¹). As shown in Figure 3C, the LCST dropped considerably, tending towards an appropriate temperature range. With the increase in concentration from 6 to 20 gL⁻¹, the LCST of poly(DMA-GA-2%) decreased from 59 to 45°C, which was the same as the variation tendency of poly(DMA-GA-1%). From this, it was apparent that the thermoresponsive properties of poly(DMA-GA-x%) were sensitive to both the GA content and the variation in concentration. The sensitivity was ascribed to the remarkably hydrophobic nature and rigid skeleton of GA moieties. In addition, the phase transition of this polymer was reversible upon cooling, despite the small hysteresis, as shown in the Supporting Information. The hysteresis was caused by the extra interchain hydrogen bonding formed in the collapsed state at higher temperature.[10] Because the phase transition of poly(DMA-GA-2%) at 10 gL⁻¹ was sharp enough and the LCST was not high, we used this concentration in the following experiments.

**Figure 4.** Transmittance variation of the aqueous solution of poly(DMA-GA-2%) (10 gL⁻¹, [GA units] = 1.47 mmolL⁻¹) as a function of temperature observed at a wavelength of 500 nm and a heating rate of 1°Cmin⁻¹: A) in the presence of different molar ratios of [β-CD to GA]; B) [β-CD]/[GA] = 5 and in the presence of different amounts of Na-Ad; C) in the absence of any additives and at molar ratio of [β-CD]:[Na-Ad]/[GA] = 5 before and after dialysis.

**Tunable Thermoresponsive Behavior Induced by the Host-Guest Interaction between GA and β-CD**

In our previous study,[7a] we reported a 1:1 inclusion complexation of GA with β-CD (the binding constant \(K_b = 1.59 \times 10^7 \text{M}^{-1}\)). In addition, the \(^1H\) NMR spectrum further confirmed the complexation of β-CD with the polymer containing GA pendants. Very broad peaks of methyl groups of the GA pendants were observable in D₂O (Figure 2A), which indicated the formation of aggregates by sequestration of hydrophobic GA units within hydrophilic polydimethylacrylamide. On addition of β-CD, signals for methyl protons (positions 23–28) became sharp and shifted downfield (Figure 2B), because the polymer became more hydrophilic upon the complexation of GA with β-CD. Hence, it is expected that this inclusion complexation can be used to tune the thermoresponsive properties of poly(DMA-GA).

Owing to the host-guest interaction between GA pendants and β-CD, poly(DMA-GA) formed a side-chain polypseudorotaxane after addition of β-CD, as shown in Figure 1. We studied the influence of β-CD on the LCST of a poly(DMA-GA-2%) aqueous solution. Figure 4A displays the LCST curves of this polymer aqueous solution after mixing 1.66, 3.33, 4.99, 6.66,
Consequently, a larger amount of [GA units] Size distributions of aqueous solution of poly(DMA-GA-2%) at [GA units] with [GA units] was more than 1:1, which may be attributed to two reasons: 1) the GA pendants were difficult to access, which hindered the complex formation compared with free guest molecules; 2) in our previous work, isothermal titration calorimetry (ITC) experiment results showed that the binding constant ($K_b$) between sodium glycyrrhetinate (GA-Na) and β-CD was $1.59 \times 10^3 \text{M}^{-1}$, thereby suggesting this host–guest interaction was not strong enough. Actually, $K_b$ is reduced by one order of magnitude when the guest group is bound to a polymer backbone.1) Consequently, a larger amount of β-CD was needed to saturate the GA pendants. Evidently, the extra addition of β-CD to guest molecules could shift the equilibrium in the direction of complexation, which led to a continuous improvement of the LCST. As shown in the inset of Figure 4A, the LCST steadily increased with further addition of β-CD.

To realize the reversible adjustment of the LCST, we further introduced a competing guest, sodium 1-adamantylcarboxylate (Na-Ada), to investigate the effect on the LCST of poly(DMA-GA-2%) aqueous solution. With addition of Na-Ada into poly(DMA-GA-2%) aqueous solution in the presence of β-CD (5 equiv), a gradually reversible decrease of the LCST was observed, as shown in Figure 4B. Owing to the higher binding constant of Na-Ada12) with β-CD ($K_b = 3.95 \times 10^4 \text{M}^{-1}$) than GA pendants, the decrease of the LCST indicated that Na-Ada replaced the GA as the main guest to enter the cavities of β-CD, which reversibly tuned the thermoresponsive properties of the GA-containing copolymers. The LCST had a similar value to that of pure polymer without β-CD, which suggested that Na-Ada has taken the place of almost all of GA as the main guest. The $^1$H NMR spectrum further confirmed the reversible transition. On addition of Na-Ada, signals for methyl protons (positions 23–28) broadened reversibly and shifted upfield, which is almost the same as the case of pure polymer without β-CD (Figure 2C). Above all, we accurately adjusted the LCST of the thermoresponsive polymer in any range (around 15 °C) by means of controlling the dosage of host (β-CD) and competing guest (Na-Ada). Meanwhile, the transmittance-temperature curves became sharper than that in the presence of mere β-CD. Additionally, the complexes composed of Na-Ada with β-CD may further aggregate with the polymer to form much larger aggregates, resulting in sharper phase transitions. To prove this speculation, we removed the complexes of Na-Ada with β-CD by dialysis. As shown in Figure 4C, after removal of the complex, the transmittance-temperature curves become broader, closely resembling that of pure polymer without β-CD.

Investigation of the Size and Morphology of Polymeric Aggregates

The effect of β-CD on the aggregation behavior of poly(DMA-GA-2%) was investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). We observed that the poly(DMA-GA-2%) formed typical nanoparticles with a size around 30 nm at 25 °C, below the LCST (Figure 5A, B, and 6A). Upon heating the solution to 60 °C, above the LCST, stable globules formed and the size of aggregates increased on average to 265 nm (Figures 5C, D, and 6B), because the polymer backbone of DMA underwent a phase transition from random coils to condensed globules, in accordance with a previous thermoresponsive investigation. After β-CD was added into the aqueous solution of poly(DMA-GA-2%) at 25 °C, the size of the aggregates changed slightly from 30 to 28 nm (Figure 6C and the Supporting Information). Whereas, after the temperature...
was raised to 70 °C (Figure 6D and the Supporting Information) showed the size of aggregates was 237 nm, clearly smaller than those formed by pure copolymers above the LCST. This was because GA pendants and β-CD formed a CD-threaded side-chain polysepdorotaxane, and subsequently the hydrophilic exterior of β-CD improved the copolymer solubility in water, which hindered the further aggregation of the globules.[11]

To confirm the role of the polymer backbone of DMA on the host–guest interactions between β-CD and GA pendants, a control experiment was conducted, in which the aggregate size of poly(DMA) was measured before and after the addition of β-CD (see the Supporting Information). There was no obvious changes in size and morphology in the presence of β-CD, which indicated that the polymer backbone of DMA had no significant influence on the complexation between β-CD and the GA pendants. This result was in agreement with reports that the physical mixing of β-CD with poly(DMA) caused no complex formation.[13,14]

Furthermore, the effect of Na-Ada, as the competing guest molecule, was also studied by TEM images and DLS. In the presence of Na-Ada at 25 °C, below the LCST, nanoparticles slightly grew up to 35 nm (Figure 6E and see the Supporting Information), which was a little larger than those formed by pure copolymers under the same conditions. When the aqueous solution was heated above the LCST, the size of the aggregates dramatically increased to 315 nm, as shown in Figure 6F and the Supporting Information. It indicates that the formation of the larger aggregates becomes easier, since the complexation of Na-Ada with β-CD leads to restoration of the free polymer chains. Therefore, we re-measured the size of the aggregates after removal of the complex by dialysis. The size was 31 nm below the LCST whereas it was 270 nm above the LCST, similar to those of pure polymers (Figure 6G,H, and the Supporting Information). The small difference in size below the LCST should be attributed to the change in the molecular-weight distribution after dialysis.

**Conclusion**

This study showed that the LCST behavior of glycyrrhetinic acid (GA)-containing copolymers can be remarkably adjusted over broad temperature ranges by changing the concentration and the content of GA pendants, which actually remained quite low (just 1–4 mol%). By complexing β-CD with GA pendants, a side-chain polysepdorotaxane was obtained, and the LCST of the copolymer was raised significantly upon gradual addition of β-CD. Moreover, a precisely reversible control of the LCST behavior of poly(DMA-GA) was realized by adding Na-Ada as the competing guest. Clearly, by utilizing the novel biocompatible β-CD/GA host–guest pair, the LCST behavior of the thermoresponsive copolymer was effectively tuned. Such a tuning may be useful to understand the effects of the host–guest β-CD/GA pair on the thermoresponsive process and helpful to design the smart biomaterials based on natural molecules.

**Experimental Section**

**Materials**

Methacryloyl chloride, [β]-cyclodextrin ([β]-CD), glycyrrhetinic acid (GA), 1-adamantanecarboxylic acid, and other reagents were local commercial products and used as received. 2,2'-Azoisobutyronitrile (AIBN) was recrystallized twice from ethanol. N,N-Dimethylacrylamide (DMA) was distilled before use.

**Instruments**

The molecular weight and polydispersity index (PDI) of the polymers were determined by gel permeation chromatography (GPC) and measured on a Waters system equipped with a high-performance liquid chromatography (HPLC) pump (Waters 1515) and a refractive index detector (Waters 2414). THF was used as the eluent at a flow rate of 1.0 mL min⁻¹ at 30 °C, and polystyrene standards were employed for the GPC calibration. 1H and 13C NMR spectroscopy experiments were carried out on a Bruker AVANCE III HD400 spectrometer at 25 °C. The lower critical solution temperature (LCST) of the samples in aqueous solutions was determined on an optical transmittance on a Cary 100 UV/Vis spectrophotometer equipped with a temperature-controlled sample controller. Polymer aqueous solutions were normally heated at a rate of 1.0 °C min⁻¹ and the LCST was taken as the middle point of the transmittance change observed at 500 nm. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano S90 instrument and the scattering angle was fixed at 90°. Transmission electron microscopy (TEM) images were recorded on a Hitachi H-7650B electron microscope operating at the accelerating voltage of 80 kV. The samples were prepared by dropping the sol (10 μL) on the carbon-coated copper grid and allowed to stand for 1 min. Then the excess sample solution was wiped away by filter paper, and dried in air for 24 h.

**Acknowledgements**

This work is supported by the NSFC (no. 21472108, 21604085, 21674022), National Key Research and Development Plan (no. 2017YFD0200302), and Jilin Science Foundation for Youths (no. 20160520135JH). J. H. and Q. Y. thank the support of the State Key Laboratory of Molecular Engineering of Polymers, Fudan University (K2017-26) and the State Key Laboratory of Polymer Physics and Chemistry, CIAC (201627).

**Keywords:** cyclodextrins - host–guest systems - natural products - polymers - thermoresponsive

Manuscript received: April 17, 2017
Revised manuscript received: June 1, 2017
Accepted manuscript online: June 13, 2017
Version of record online: July 7, 2017