Thermoresponsive Supramolecular Dendrimers via Host–Guest Interactions

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A series of first- and second-generation supramolecular dendrimers (SDs) was prepared via host–guest interaction from the cyclodextrin trimer and oligoethylene glycol (OEG)-based threefold dendritic guests. These SDs are water soluble at room temperature but show characteristic thermoresponsive behavior at elevated temperatures. Their thermoresponsiveness was investigated by UV–Vis spectroscopy and dynamic light scattering measurements. Supramolecular complexation from a mixture of two dendritic guests with different hydrophilicity was performed to check the feasibility to tune the phase transition temperatures of codendrimers. Based on temperature-varied 1H NMR spectra, it was found that the SDs start to decompose when the OEG units are dehydrated.

1. Introduction

Dendrimers are one of the most attractive macromolecules in nanotechnology and biomedical science due to their structural complexity, monodispersity, nanometer size, and well-defined globular shapes.1 They are considered to be a “dendritic box” and can encapsulate small molecules. Up to date, various types of dendrimers with different core, branch units, and peripheries have been reported.2 One intriguing class of dendrimers is these with stimuli-responsive properties, which can undergo physical changes upon external–internal stimuli. These properties can not only enrich the functionalities of dendrimers but also may offer easy way to control these nanocapsules “on” or “off” by external stimulus.

Many efforts have been paid to develop dendrimers responsive to solution pH, light, or temperature.3 Among them, thermoresponsive dendrimers have attracted considerable attention and can be prepared through several strategies. This kind of dendrimers are soluble in water at low temperature, but their aqueous solutions undergo entropy-driven phase transitions from homogeneous into heterogeneous upon heated above the lower critical solution temperature (LCST). A traditional synthetic strategy is to introduce thermoresponsive polymer chains, such as poly(N-isopropylacrylamide)s, to the periphery of dendrimers via grafting-from technique.4 It is unavoidable that the well-defined structure of the dendrimer will be destroyed by the attachment of polydispersed polymer chains. To avoid this, Kono and coworkers5 reported the preparation of thermoresponsive dendrimers by the introduction of hydrophobic units, such as isobutyramide, alkyl amide, or phenylalanine, to the periphery of dendrimers via grafting-from technique.6 Another strategy for the preparation of thermoresponsive dendrimer is to incorporate hydrophilic and hydrophobic units with appropriate ratio within the dendrimers.7,8 We recently reported a series of dendronized polymers and dendrimers based on threefold oligoethylene glycol (OEG), which show unprecedented thermoresponsive behavior with sharp phase transitions.
and small hystereses, and their phase transition temperatures covered the range of 27–64 °C depending on mainly the peripheral groups of the dendrons. More importantly, OEG-based dendrimers are nontoxic to cells, which may find usages in bio-related areas.\[7\] Although several kinds of thermoresponsive dendrimers were prepared and their thermoresponsive behaviors investigated, they are all constructed through covalent linkage, and prepared by either divergent or convergent approach. The tedious synthesis remains a major drawback for this class of promising macromolecules. Once the dendrimers were prepared, it will not be possible to change the covalently fixed structures or architectures. Therefore, it remains an important challenge to develop novel strategies for preparing thermoresponsive dendrimers with easy synthesis and dynamic properties.

Alternatively, the supramolecular self-assembly of dendritic building blocks provides an easy strategy to prepare noncovalent dendrimers with versatile structures.\[8\] Several approaches have been developed for the assembly of supramolecular dendrimers (SDs) in bulk, organic media, or water, which takes advantage of highly directional noncovalent interactions, such as metal coordination,\[9\] hydrogen bonds,\[10\] π-π stacking,\[11\] ionic bonds,\[12\] host–guest interactions,\[13\] or even mechanical interlocked bonds.\[14\] Although SDs have been widely reported, less attention has been paid on the preparation of SDs with thermoresponsiveness.\[15\] Recently, we reported a series of supramolecular dendronized polymers through the host–guest interaction from linear polymer with cyclodextrin pendants and OEG-based dendritic guests cored with adamantyl group.\[16\] These supramolecular dendronized polymers show characteristic thermoresponsive behavior as their covalent counterparts, but are much easier to have tunable phase transition temperatures, which can be realized by just mixing guests of different hydrophilicities to complex with the same polymer host. Encouraged by this, we present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}) with the same polymer host. Encouraged by this, we present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}). We present here the investigation on the construction of SDs via host–guest interaction from a cyclodextrin trimer (CD \textsubscript{3}).

**Figure 1.** Chemical structures of the dendritic guests (EtG1-Ada, MeG2-Ada, EtG2-Ada), and the cyclodextrin trimer host (CD \textsubscript{3}).

2. Experimental Section

2.1. Materials

Dendritic guests (EtG1-Ada, MeG2-Ada, EtG2-Ada) were synthesized according to our previous reports.\[14\] Mono-6-deoxy-6-amino-β-CD \textsubscript{2} was prepared according to a literature method.\[17\] Triethylamine (TEA) was dried over NaOH pellets. Dichloromethane (DCM) was distilled from CaH \textsubscript{2} for drying. Pure water was redistilled. Other reagents and solvents were purchased at reagent grade and used without further purification. All syntheses were carried out under a nitrogen atmosphere. Macherey-Nagel-precoated thin-layer chromatography (TLC) plates (silica gel 60 G/UV254, 0.25 mm) were used for a TLC analysis. Silica gel 60 M (Macherey-Nagel, 0.04 = 0.063 mm, 200 = 300 mesh) was used as the stationary phase for column chromatography.

2.2. Instrumentation and Measurements

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded on a Bruker AV 500 (\textsuperscript{1}H: 500 MHz, \textsuperscript{13}C: 125 MHz) spectrometer. High resolution MALDI–TOF–MS analyses were performed on IonSpec Ultra instruments. UV–Vis turbidity measurements were carried out for cloud point \(T \textsubscript{c}\) determination on a PE UV–Vis spectrophotometer Lambda 35 equipped with a thermostatically regulated bath. Aqueous polymer solutions were placed in the spectrophotometer (path length 1 cm), and heated or cooled at a rate of 0.2 °C min \(^{-1}\). The absorptions of the solution at \(λ = 500\) nm were recorded every 5 s. The \(T \textsubscript{c}\) is determined the one at which the transmittance at \(λ = 500\) nm had reached 50% of its initial value. Dynamic light scattering (DLS) measurements were performed on the DynaPro Nanostar instrument (Wyatt Technology Corporation, He–Ne laser, \(λ \textsubscript{d} = 658\) nm).
2.3. Synthesis of CD₃

1,3,5-Benzene tricarbonyl trichloride (0.50 g, 1.88 mmol) was added into a solution of pentafluorophenol (1.35 g, 7.33 mmol), TEA (0.76 g, 7.51 mmol), and DMAP (10.00 mg) in DCM (50 mL) at −5 °C and then the reaction temperature was elevated to room temperature. After stirring for 3 h, the solution was washed with brine three times. The organic phases were combined and dried over MgSO₄. After filtration, purification by column chromatography with DCM afforded the compound 1 (1.30 g, 97%) as a white solid. This compound (0.86 g, 1.22 mmol) was added into the mixture of compound 2 (5.10 g, 4.50 mmol) and disopropylethylamine (DiPEA, 1.14 g, 8.82 mmol) in DMF (50 mL) at −15 °C. After stirring for 2 h, the reaction mixture was allowed to rise to room temperature and stirred for another 24 h. Precipitation in acetone afforded the raw product, which was dialyzed against deionized water (MWCO 1000) for 2 days. The title product was freeze-dried into a white product, which was dialyzed against deionized water (MWCO 1500) for 2 days.

2.4. Characterization

HRMS (ESI) m/z: [M + Na]⁺ calcd for C₁₅₁H₂₁₃N₂O₁₂0Na, 3579.1318; found, 3579.8182.

3. Results and Discussion

3.1. Supramolecular Formation

The SDs were constructed by a host-carrying three β-CD moieties and the OEG-based dendritic guests. Synthesis of the cyclodextrin trimer host (CD₃) was outlined in Scheme 1. Starting from 1,3,5-benzenetricarbonyl trichloride, reaction with pentafluorophenol in the presence of TEA afforded the corresponding pentafluorophenol active ester 1 in an excellent yield (97%). Coupling of this active ester with mono-6-deoxy-6-amino-β-CD (2) yielded the aimed cyclodextrin trimer CD₃, which was characterized by ¹H and ¹³C NMR spectroscopy (Figure S1, S2, Supporting Information) as well as high-resolution mass spectrometry.

When mixing the dendritic guests (EtG₁-Ada, MeG₂-Ada, or EtG₂-Ada) with the trimer CD₃ in aqueous solutions with molar ratio [CD₃]/[dendritic guest] = 1:3, SDs were supposed to be formed through the strong inclusion interaction between the cyclodextrin and adamantane moieties. In the following, the complexation of the trimer CD₃ with the more hydrophobic EtG₂-Ada is selected as an example. ¹H NMR spectroscopy was applied to prove the inclusion complexation, and the typical spectra of EtG₂-Ada, CD₃, and their equivalent (based on CD and adamantyl moieties) mixture are shown in Figure 2. When mixing EtG₂-Ada with CD₃ in D₂O, the heights of the proton signals from both of the host and guest molecules decrease slightly, but most of the signals are still well resolved, indicating the typical behavior of molecules with low molar masses. After complexation, the signals from adamantyl moieties at about δ = 1.48 and 1.80–1.90 (Figure 2a) were shifted downfield to δ = 1.66 and 2.00–2.20 (Figure 2b), respectively. The shifts for inner proton signals of C(3)–H and C(5)–H from cyclodextrin moieties can not be recognized as they overlapped with the signals from OEG units of the dendron. Instead, 2D-NOESY NMR spectroscopy was applied, and the spectrum shows correlations between the guest signals and the host signals.
EtG2-Ada and CD, and Me-SD2 from MeG2-Ada and CD) are all well soluble in water at room temperature, but their clear aqueous solutions turn into opaque at elevated temperatures. UV–Vis spectroscopy was then applied to follow their phase transition processes and the turbidity curves are plotted in Figure 4. The turbidity curves from the guests are included for comparison. Both of the second generation SDs Et-SD2 and Me-SD2 show quite sharp phase transitions (<1.5 °C) and small hysteresis (around 1 °C). In contrast, the first-generation SD Et-SD1 shows much broader transition and quite abnormal reversible behavior. Its rehydration in cooling process is even ahead of its dehydration in heating process, which suggests significant amount of aggregates precipitated during the heating process. The \( T_c \)s of the SDs Et-SD1, Et-SD2, and Me-SD2 were determined to be 63.3, 43.9, and 72 °C, respectively. [16] EtG1-Ada shows much lower hydrophilicity than EtG2-Ada due to relatively larger proportion of hydrophobic adamantyl moiety within the molecule. Above results demonstrate that: (1) the hydrophilic CD moieties at the core of the SDs contributes significantly to the thermally induced phase transitions, especially for the smaller first-generation dendrimer (Et-SD1); (2) the methoxyl-terminated SDs show more hydrophilic than the

### 3.2. Thermoresponsive Behavior

Supramolecular dendrimers formed from the first- and second-generation dendritic guests with cyclodextrin trimer CD, EtG1-Ada and CD, EtG2-Ada and CD, were investigated in detail, and the spectra from CD, and EtG2-Ada are summarized in Figure 3. Upon addition of 0.1 equivalent CD, into the aqueous solution of EtG2-Ada, the characteristic proton signals from adamantyl moieties between \( \delta = 1.4–1.8 \) were shifted downfield significantly, while protons from OEG units kept their chemical shifts unchanged in spite of the slight decrease of their intensities. With the successive addition of CD, the chemical shifts of adamantyl proton signals were gradually shifted downfield and tended to remain unchanged when the host–guest molar ratio reached 0.33. The molar ratio plot indicates the 1:3 stoichiometry between the cyclodextrin trimer host and dendritic guests within experimental error (Figure S5, Supporting Information).

![Figure 3. \( ^1 \text{H} \) NMR spectra of the complex from EtG2-Ada and CD, with different molar ratios.](image)

![Figure 4. Plots of transmittance versus temperature for 0.25 wt% aqueous solution EtG2-Ada, Et-SD1, Et-SD2, MeG2-Ada, and Me-SD2.](image)
was investigated by UV–Vis spectroscopy (Figure 6). As expected, all these codendrimers showed quite sharp transitions, and the $T_c$ increased as the proportion of more hydrophilic guest MeG2-Ada increased. However, the variability is very small, and the $T_c$s of these codendrimers are much closer to that of Et-SD2. Considering the great dependence of $T_c$s on concentration of these SDs, the change of the $T_c$s here is possibly caused by the dilution of Et-SD2 upon mixing with the Me-SD2 solution. This result indicates that the thermoresponsive behavior of supramolecular codendrimers was mainly dominated by the more hydrophobic dendritic guest (EtG2-Ada), which suggests the cooperative effect between dendritic guests during dehydration and collapse is negligible. This phenomenon is quite different from the situations for the supramolecular dendronized copolymers formed from the same dendritic guests. In the latter case, macromolecular effects play a major role to show an efficient cooperative effect, thus make it possible to tune the phase transition temperatures by changing the ratio of the dendritic guests with different hydrophilicities.

The aggregation process of supramolecular dendrimers Et-SD2 during the thermally induced phase transitions was followed by DLS measurements (Figure S7, Supporting Information). Below the $T_c$ (51.6 °C for 0.049 wt% solution), the $T_c$ of Et-SD2 increases significantly from 38.2 to 50.2 °C. This indicates the $T_c$s of supramolecular dendrimers are much more dependent on the solution concentration than that of the supramolecular dendronized polymers (the $T_c$ change is only 3 K with the same concentration change).\cite{20} The effect of salt addition on the thermoresponsiveness of supramolecular dendrimers was further examined (Figure S5, for turbidity curves see Figure S6, Supporting Information). The $T_c$ decreases linearly with the increase of salt concentration, while the phase transitions are still kept sharp and fast in the presence of NaCl with different concentrations.

![Figure 5. Cloud point dependence on concentrations of Et-SD2 and NaCl.](image)

One unique feature of supramolecular constructions is the structure variability due to the dynamic nature. Therefore, we tried to construct supramolecular codendrimers from two dendritic guests with different hydrophilicities, to see whether it is possible to tune their phase transition temperatures of supramolecular codendrimers by simply changing the ratios of the two guests. The typical supramolecular codendrimers formed from the mixture of EtG2-Ada/MeG2-Ada at molar ratios of 3:1, 1:1, and 1:3 were prepared, and their thermoresponsive behavior was investigated by UV–Vis spectroscopy (Figure 6). As expected, all these codendrimers showed quite sharp transitions, and the $T_c$ increased as the proportion of more hydrophilic guest MeG2-Ada increased. However, the variability is very small, and the $T_c$s of these codendrimers are much closer to that of Et-SD2. Considering the great dependence of $T_c$s on concentration of these SDs, the change of the $T_c$s here is possibly caused by the dilution of Et-SD2 upon mixing with the Me-SD2 solution. This result indicates that the thermoresponsive behavior of supramolecular codendrimers was mainly dominated by the more hydrophobic dendritic guest (EtG2-Ada), which suggests the cooperative effect between dendritic guests during dehydration and collapse is negligible. This phenomenon is quite different from the situations for the supramolecular dendronized copolymers formed from the same dendritic guests. In the latter case, macromolecular effects play a major role to show an efficient cooperative effect, thus make it possible to tune the phase transition temperatures by changing the ratio of the dendritic guests with different hydrophilicities.

The aggregation process of supramolecular dendrimers Et-SD2 during the thermally induced phase transitions was followed by DLS measurements (Figure S7, Supporting Information). Below the $T_c$ (51.6 °C for 0.049 wt% solution), the hydrodynamic radius ($R_h$) of Et-SD2 is very small (~2 nm). As the temperature reached 51 °C, large aggregates were formed, and the $R_h$ increased abruptly to about 750 nm. With the further increase of temperature, the size of the aggregate increased accordingly, and reached several micrometers. This aggregation behavior is quite similar to that of the covalent OEG-based dendrimers reported previously.\cite{20}
3.3. Thermally Induced Decomposition

It has been documented that the decomplexation of the supramolecular complex or polymers takes place during their phase transitions. \[^{[16,21]}\] Herein, the decomplexation process of these SDs was investigated by temperature-varied \(^1\)H NMR spectroscopy. The partial spectra (1.0–2.7 ppm) of Et-SD2 are shown in Figure 7a (for full spectra see Figure S8, Supporting Information). All proton signals from EtG2-Ada are time averaged at low temperature, but split into two groups as the temperature increased to 36 °C, which is just below the phase transition temperature (38.2 °C at the used concentration). The new split signals (up-field) are related to the decomposed (free) species. This tendency for these proton signals to split is enhanced with increase of the solution temperature. By comparing the signal intensities of the complexed and decomposed species, the percentage of decomplexation during the phase transition can be calculated, and the results are plotted in Figure 7b. The decomplexation starts around 36 °C with a percentage of decomposed species around 20%, which increased stepwisely to 85% at 50 °C. Similar phenomena were also observed for Me-SD2 (Figure S9, Supporting Information), but its decomplexation took place at around 56 °C, which is far above the decomplexation temperature for Et-SD2, but below the phase transition temperature of Me-SD2 (64 °C at the used concentration). These results demonstrate that: (1) originating from the steric hindrance of dehydrated OEG-based dendrons, the complexation kinetics of the cyclodextrin with the dendritic guests becomes a slow exchange process relative to the NMR timescale once the thermally induced dehydration occurs\[^{[22]}\]; (2) the thermally induced dehydration of OEG-based dendrons induces the decomplexation of the supramolecular dendrimers, thus, the more hydrophobic dendritic guest decomplexes at a much lower temperature than that of the more hydrophilic guest.

4. Conclusion

A series of first- and second-generation SDs was constructed via host–guest interaction from the cycloextrin timer CD\(_3\) and OEG-based dendritic guests. These SDs show characteristic thermostresponsive behavior when heated to elevated temperatures. Their phase transition temperatures can be varied from 43 to 72 °C by changing either the periphery units or the generation of the dendritic guests. Besides, the second-generation SDs exhibit much sharper phase transitions and smaller hystereses compared with the first-generation one. Temperature-varied \(^1\)H NMR spectroscopy shows the thermally induced dehydration of OEG-based dendrons causes the decomposition of SDs, and the more hydrophilic dendritic guest decomposes at a much higher temperature than that of the more hydrophobic one.
Interestingly, this decomposition does not show obvious influence on their thermoresponsiveness. When compared to their supramolecular dendronized (co)polymer counterparts formed from the same components, these SDS show much higher $T_c$, and their thermoresponsive behavior is more dependent on the solution concentrations and salt addition. Furthermore, the $T_c$ of the codendrimers from a mixture of guests with different hydrophilicity are dependent mostly on the $T_c$ of more hydrophobic one, but less on the ratio of the guests. All these results are indicative of absence of significant cooperative effects between neighboring dendrons among the SDs during the thermally induced dehydrophation, which should be originated from the different architecture effects of dendrimers and dendronized polymers.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements: We thank Dr. Hongmei Deng (Instrumental Analysis and Research Center of Shanghai University) for her assistance in NMR measurements. This work is financially supported by National Natural Science Foundation of China (Nos. 21034004, 21104043, and 20974020) and the Science and Technology Commission of Shanghai (No. 10520500300 and 11pj1404100).

Received: June 12, 2012; Revised: July 10, 2012; Published online: August 10, 2012; DOI: 10.1002/macp.201200320

Keywords: cyclodextrins; dendrimer; host–guest interaction; supramolecular chemistry; thermoresponsive polymer