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Tuning Thermoresponsive Supramolecular G-Quadruplexes

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Abstract

Thermoresponsive systems are attractive due to their suitability for fundamental studies as well as their practical uses in a wide variety of applications. While much progress has been achieved using polymers, alternative strategies such as the use of well-defined nonpolymeric supramolecules are still underdeveloped. Here we report three 8-aryl-2'-deoxyguanosine derivatives (8ArGs) that self-assemble in aqueous media into precise thermoresponsive supramolecular G-quadruplexes (SGQs). We report the synthesis of such derivatives, studies of their isothermal self-assembly, and the thermally induced assembly to form higher-order meso-globular assemblies we term supramolecular hacky sacks (SHS). The lower critical solution temperature (LCST) that indicates the formation of the SHS was modulated by changing (a) intrinsic parameters (i.e., structure of the 8ArGs); (b) extrinsic parameters such as the salt used to promote the formation of the SGQ; and (c) supramolecular parameters such as the coassembly different 8ArGs to form heteromeric SGQs. Changes in the intrinsic parameters lead to LCST variations in the range of 28–59 °C. Modulating extrinsic parameters such as replacing KI with KSCN abolishes the thermoresponsive phenomenon whereas changing the cation from K^+ to Na^+ or adjusting the pH (in the range of 6–8) has negligible effects on the LCST. Modulating supramolecular parameters results in transition temperatures that are intermediate between those obtained by the respective homomeric SGQs, although the specific proportions of the subunits are critical in determining the reversibility of the process. Given the extensive applications of thermoresponsive polymers, the nonpolymeric supramolecular counterparts presented here may represent an attractive alternative for fundamental studies and biorelevant applications.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information

Details of the synthesis and characterization of **1–3**. 1D and 2D 1H NMR for the self-assembly studies, including details for other realized experiments such as turbidimetry, DLS, and DSC. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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INTRODUCTION

The development of artificial stimuli-responsive (i.e., smart) materials has been driven by both fundamental and applied research^{1,2} including catalysis,^{3,4} nanotechnology,^{5,6} and drug delivery,⁷ among others. These materials are characterized by their ability to undergo significant changes (e.g., shape and/or conformation) in a property triggered by a relatively small stimulus (e.g., physical or chemical).^{8,1,2} For example, in thermoresponsive materials, when amphiphilic compounds are made increasingly hydrophobic, before becoming completely insoluble, they can reach a range of compositions where a small increase in temperature leads to the phenomenon of lower critical solution temperature (LCST).⁹ Above the LCST, if the phenomenon occurs in solution, then colloidal suspensions of nano/microglobules are formed, and when this occurs in macroscopic hydrogels, it leads to significant reductions in volume.¹⁰ These characteristics have made thermoresponsive materials attractive because of the insight they contribute to fundamental questions such as the origin of multiple hydrophobic phenomena^{11–13} and can be used as nano/microenvironments for multiple applications.^{1,14}

Most substances that show the LCST phenomenon are amphiphilic polymers, although some small molecules such as triethylamine¹⁵ and nicotine¹⁶ are also known to show such a property. The hydrophobic/hydrophilic ratio of amphiphilic polymers can be readily tuned to obtain a desired transition temperature.² Upon reaching the transition temperature, polymers undergo a coil-to-globule transition that is frequently followed by their aggregation or phase transition.^{17,18} Thermally responsive polymers, most notably, elastin-like polypeptides¹¹ and poly(*N*-isopropylacrylamide) (pNIPAm),¹⁹ are attractive due to their suitability for fundamental studies as well as their practical uses in many applications such as medicinal chemistry.^{2,8,20,21} Thermoresponsive dendrimers, hyperbranched monodisperse polymers,²² have also been reported²³ to have properties similar to linear polymers.

Supramolecular self-assembly offers a complementary biomimetic strategy to the use of polymeric systems for the development of functional nanostructures. In principle, non-polymeric, well-defined supramolecular assemblies could be developed to show the LCST phenomenon. However, until our recent discovery,²⁴ this strategy was yet to be reported.²⁵ In recent years, various groups^{26,27} including ours have identified guanosine (G) and some of its derivatives as excellent recognition motifs for the construction of functional nanostructures. Guanosine and related compounds have a propensity to form planar tetramers (G-tetrads, Figure 1), which are held together by hydrogen bonds and metal cation complexation such as K⁺ or Na⁺. Further stacking of such tetrads leads to the formation of supramolecular G-quadruplexes (SGQs). These characteristics confer guanine with the ability to self-assemble into SGQs as the free base or as part of nucleosides, nucleotides, or oligonucleotides.²⁸ Our specific efforts are aimed at studying the self-assembly of 8-aryl-2'-deoxyguanosine derivatives (8ArGs). We have explored how the 8-aryl group can be used to modulate the properties of the resulting precise supramolecular structures (Figure 1).

We have developed the 8-*meta*-acetyl-guanosine (mAG) moiety into an attractive recognition motif to enable the construction of well-defined and discrete supramolecular nanostructures.²⁹ For example, we used the mAG moiety to construct lipophilic

hexadecameric self-assembled dendrimers (SADs).³⁰ Our discovery that a positively charged hydrophilic mAG derivative self-assembles isostructurally into hexadecamers in aqueous media³¹ prompted us to explore the construction of congeneric hydrophilic SADs.³² Our laboratory developed an 8-*meta*-acetyl-guanosine (mAG) derivative **1** that self-assembles in aqueous media into a precise (discrete and well-defined) hexadecamer, SGQ **1**₁₆ (Figure 1a).²⁴ Furthermore, during such studies, we discovered that SGQ **1**₁₆, exhibited the LCST phenomenon upon reaching a temperature of 58 °C, which triggered the formation of higher-order nano/microglobular assemblies we term here supramolecular hacky sacks (SHS) (Figure 1b). In this report, we demonstrate that modulating parameters that are intrinsic (i.e., structural information in the molecular 8ArG subunits), extrinsic (e.g., cation, anion, and pH), and supramolecular (e.g., coassembly of two subunits) provide multiple strategies to control the thermally induced assembly of SGQs and the properties of the corresponding SHS.

Here we expand the scope of thermoresponsive SGQs (i.e., that show the LCST phenomenon) made from two additional 8-*meta*-carbonyl-2'-deoxyguanosine derivatives (mCGs) that are similar to mAG and self-assemble into well-defined hexadecamers in aqueous media. Differential scanning calorimetry (DSC) and dynamic light scattering (DLS) were used for the detailed study of their temperature-triggered assembly. These findings further illustrate how principles of supramolecular design provide a new paradigm for the development of smart thermoresponsive materials with properties and applications complementary to those of covalent polymers.²

EXPERIMENTAL SECTION

NMR Studies

The self-assembly of mCGs was assessed using a Bruker DRX-500 NMR spectrometer equipped with a 5 mm BBO probe. In water, a conventional 1D presaturation pulse sequence with the excitation pulse set over the water peak at 4.7 ppm was used. A standard proton sequence was used for experiments in D₂O. Self-assembly studies were performed, for example, using a 10 mM solution of **1** in 650 µL of H₂O–D₂O (9:1, potassium buffer, 2 M KI). For the NOESY experiment, a phase-sensitive 2D NOESY pulse sequence with presaturation (noesyphpr) from Bruker was used. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-*d*₄ (Aldrich) was used as the internal standard for the NMR experiments performed in H₂O/D₂O (9:1). All NMR experiments were performed at 298.2 K unless otherwise stated.

Turbidimetry (Cloud-Point) Studies

These measurements were performed at 500 nm using a Varian UV-visible spectrometer (model Cary Bio-100). The heating rate was adjusted to 2.0 °C/min using a Cary temperature controller apparatus from Varian. Derivatives **1–3** (all at 10 mM with 2 M KI) were dissolved in a potassium buffer (pH 7.4) and filtered with a 0.45 µm Nylon filter using Fisherbrand 10 mm o.d. glass tubes prior to the experiment. All of the transmittance measurements have an error of ±1 nm based on their standard deviation of 10 measurements.

DSC Studies

These measurements were performed on a VP-DSC microcalorimeter (MicroCal Inc.) with a cell volume of 0.517 mL. The total heat required for the dissociation of the hexadecamer was measured in degassed solutions with PBS (pH 7.4, 2 M KI) in a temperature range of 20–95 °C using a heating rate of 1 °C min⁻¹. For each solution, the excess heat capacity curve was constructed by the subtraction of the buffer (PBS) scan from the sample scan. The deconvolution curves were generated using Origin v7; the transition is a two-step process in which we measure the temperature of SHS formation (T_M) in the right deconvoluted curve and the melting temperature of the SGQ assembly (T_m) in the left deconvoluted curve. Both values were taken as the maxima in the DSC curves. Measured enthalpy (H_{cal}) values in the microcalorimeter were obtained from the Origin v7 software, in which C_p is the heat capacity of the solution during the disassembly process.

DLS Studies

These measurements were performed using a DynaPro Titan (Wyatt Technology Corporation) with temperature control and a microsampler with a diode laser at a 90° scattering angle, a wavelength at 657 nm, and a power of 15 mW. The measurements were performed with samples (7–10 mM) dissolved in PBS at pH 7.4 in a temperature range of 25–75 °C. The samples were filtered with a 0.45 µm Nylon filter using Fisherbrand 10 mm o.d. glass tubes prior to the experiments. The obtained DLS measurements were obtained from Dynamics V6 software in the form of histograms. The graphs of average hydrodynamic diameters (D_H) as a function of temperature in Figure 4 were constructed using Kaleida graph version 3.6.5, and the size distribution histograms of Figure 5 were constructed using PRISM version 4.0.

RESULTS AND DISCUSSION

Synthesis and Self-Assembly Studies

The three mCGs (**1–3**, Scheme 1) used in this study were designed to determine the modulation of intrinsic parameters affecting the LCST. All mCGs were prepared as previously reported,²⁴ where the 8-aryl group is installed via a key Suzuki–Miyaura cross-coupling reaction.^{33,34} Starting with 8-bromo-2'-deoxyguanosine (8-BrdG),³⁵ readily prepared by the bromination of C8 in the guanine moiety and subsequent Suzuki–Miyaura cross-coupling with commercially available boronic acids, had proven successful in the synthesis of 8ArGs.³⁵ Esterification of the 5'- and 3'-hydroxyl groups with 6-bromohexanoic acid affords the various bromohexanoyl esters (8ArG-hBr), which are transformed to the corresponding azido esters by treatment with NaN₃. Subsequent reaction with alkynyl-containing dendrons^{36,30} via the copper-catalyzed Huisgen 1,3-dipolar cycloaddition (click reaction)³⁷ provided dendronized derivatives **1–3** in good yields.^{38,36} All new compounds were purified and characterized by ¹H and ¹³C NMR, electrospray mass spectrometry (ESI-MS), and IR spectroscopy (details in the Supporting Information).

Derivatives **1–3** are only partially soluble (10 mM) at room temperature in aqueous phosphate-buffered solution (pH 7.4), but upon addition of KI (2 M), the resulting SGQs become readily soluble. ¹H NMR experiments of homogeneous solutions **1–3** in H₂O–D₂O

(9:1, potassium phosphate buffer, pH 7.4) reveal signature peaks characteristic of their self-assembly into hexadecameric SGQs. The ^1H NMR spectrum shows the characteristic double set of signals corresponding to two pairs of tetrads (N^1H) in different chemical environments (Figure 2). The 2D-NOESY spectrum supports the formation of **1**₁₆, **2**₁₆, and **3**₁₆ by showing the signature cross peaks characteristic of a hexadecameric SGQ (Figure 2a).³¹ By using 2D-NOESY, we can follow the signature guanine cross-peak patterns of the N^1H and NH_2 proton signals of two pairs of tetrads (the outer and inner) in different chemical environments of the SGQ assembly in the presence of salt. These results complement the 1D water suppression ^1H NMR experiments in which we can observe two peaks corresponding to the N^1H protons that are in slow exchange with the solvent between 11 and 13 ppm, indicating the characteristic hexadecameric assembly (16 8ArG subunits). The broadness of the peaks in the ^1H NMR spectra of **2**₁₆ and **3**₁₆ is consistent with less thermodynamically stable, or more dynamic, assemblies relative to **1**₁₆. The latter is likely due to the increased steric bulk near the core of the tetrad assembly because of the larger groups attached to the *meta*-carbonyl moiety. This behavior concurs with our previous studies on the relative stability of the hexadecamers formed by the mAG (**1**) versus the mECG (**2**) scaffolds in organic media.³⁹ These findings underscore the influence of the specific 8-aryl group in the self-assembly process by tuning noncovalent interactions (e.g., π - π , CH- π , and H bonds).

LCST Modulation via Intrinsic Parameters

We performed turbidity measurements at increasing temperatures to identify the threshold for the LCST phenomenon. At room temperature (ca. 25 °C), aqueous phosphate-buffered solutions (pH 7.4, 2 M KI) of **1**₁₆, **2**₁₆, and **3**₁₆ were highly soluble. The onset of turbidity or cloud-point temperature (T_{cp}) for the solutions of **1**₁₆, **2**₁₆, and **3**₁₆ was different for each SGQ as it reflects the effective hydrophobicity of each system. As previously reported, **1**₁₆ becomes cloudy at ~59 °C; in the case of **2**₁₆ and **3**₁₆, the solution becomes cloudy at around 33 and 28 °C, respectively (Figure 3). Cooling the solution back to 25 °C restores the initial conditions (yellowish homogeneous solution), and this process can be repeated at least 10 times (Figure S10, Supporting Information) with no signs of fatigue (e.g., decomposition, insoluble aggregates).

DSC studies provide complementary quantitative information to the turbidimetry results, enabling insights into the thermodynamics of the LCST phenomenon.^{8,40,41} Figure 3 shows the endotherms obtained while heating SGQs **1**₁₆, **2**₁₆, and **3**₁₆ in aqueous phosphate buffer. The DSC for the three SGQs show broad peaks with two maxima from two overlapping peaks corresponding to the maximum of the endotherm (i.e., formation of the SHS, T_{M}),^{40,42,43} which is related to the partitioning of bound water molecules to the bulk upon heating and the thermally induced disassembly (i.e., denaturation or “melting” of SGQs, T_{m}). Both T_{M} and T_{m} decrease along the series from **1**₁₆ to **2**₁₆ to **3**₁₆ (Table 1). The changes in the former resulting from the increased effective hydrophobicity for each nanoglobular hexadecameric SGQ are associated with the remodeling of the lipophilic patches on their surfaces, as we have described recently.⁴⁴ On the other hand, the slight decrease in T_{m} is related to the increased steric repulsion within the aforementioned SGQs resulting from ethoxy (**2**), ethylamino (**3**), and methyl (**1**) groups, which destabilize the assemblies of the former two relative to the latter.³⁹

The enthalpies (H_{cal}) associated with the LCST of these SGQs were calculated from the areas under the peaks of the DSC endotherms (Table 1). The H_{cal} values for **1**₁₆ and **2**₁₆ were of the same order of magnitude (4.60 and 12.4 kcal/mol, respectively); however, those corresponding to **3**₁₆ (0.85 kcal/mol) were significantly smaller. We hypothesize that these differences in H_{cal} are due to the different interaction of the water of hydration with the relatively smaller acetyl group in **1**₁₆ when compared to either the ethoxy-carbonyl (**2**₁₆) or the ethyl-amide moieties (**3**₁₆). These structural differences can promote diverse types of hydrophobic interactions. In polymeric systems, hydrophobic interactions result from the tendency of hydrophobic molecules to minimize the interface with water molecules in the hydration shells and the corresponding entropy changes of water molecules around the hydrophobic groups. For example, it has been suggested that the phase transition of pNIPAm consists of at least two different processes: (1) a rearrangement of bound water around either hydrophobic and hydrophilic groups of pNIPAm and (2) a hydrophobic association of the lipophilic groups in the pNIPAm chains or a residual interaction between the pNIPAm side-chain residues.⁴⁵

Although the LCST determined by the onset of turbidity (T_{cp}) and the maximum in the DSC peak (T_{M}) very often coincide (Figure 3 top, for **1**), this does not have to be the case as others^{42,43} and us have previously reported.⁴⁴ The former is related to a thermally induced assembly process, and the latter is related to the partitioning of water molecules from the hydration layer to the bulk solvent. Conversely, some thermosensitive systems can also show a sub-LCST (thermally induced) assembly that occurs prior to the onset of turbidity.⁴⁶ While for **1**₁₆ the T_{cp} coincides with T_{M} , the analogous SGQs formed by **2** and **3** show differences (ΔT) of 22 and 10 °C, respectively (Table 1, Figure 3). These results are consistent with an athermal association of the SGQs, likely mediated by water molecules interacting with the hydroxy groups exposed on their surfaces.

Dynamic light scattering (DLS) studies indicate that below T_{cp} the solutions are composed of monodisperse, discrete SGQs with average hydrodynamic diameters (D_{H}) of around 5 nm (Table S1). These are in very good agreement with the values obtained from ¹H DOSY NMR experiments of 4.40 ± 0.12 nm previously reported for **1**₁₆.²⁴ Reaching T_{cp} , however, leads to the formation of large hydrated globular SHS ($D_{\text{H}} \approx 10 \mu\text{m}$, Figure 4) of relatively narrow size distributions (Figure 5), with **2**₁₆ and **3**₁₆ forming SHS that are about 10 times larger than the one formed by **1**₁₆. Continued heating above T_{cp} leads to a decrease in the average size of the SHS formed by the three derivatives being consistent with a steady dehydration, although the extent of dehydration is smaller for the SHS formed by **2**₁₆ and **3**₁₆ (Figure 4). Heating the solutions to above 70.0 °C induces the melting of the constituent hexadecameric SGQs, which in turn leads to the formation of larger aggregates of much broader size distributions (Figure 5).

LCST Modulation via Extrinsic Parameters

Adjusting the differential hydrophobicity of the system under study and its environment enables the modulation of the LCST phenomenon. This can be achieved by adjusting the balance between hydrophobic and hydrophilic groups of a given thermoresponsive system.^{11,20} For example, with polymers this is achieved by varying the composition of the

monomers during their covalent synthesis, and with the SGQs described here, by modifying the structure of the constituent 8ArG subunits. Alternatively, changes to the environment (extrinsic changes) such as adjusting the pH, the type and concentration of salts, and the ionic strength can also be employed.⁴⁷ In this way, the size and morphological features for the mesoglobules formed by thermoresponsive polymers⁴⁸ have been shown to be affected by changes in extrinsic parameters such as the temperature,⁴⁹ heating rate,⁵⁰ and nature of the ions and the corresponding ionic strength of the medium.⁵¹ Furthermore, varying the nature and concentration of ions has found applications that range from the development of isothermal ion-responsive polymers⁵² to tuning the structure, encapsulation, and release properties of nanogels.⁵³

Cation Effects

While anions generally affect the LCST of macromolecules to a greater extent than do cations,⁵⁴ the critical role of the latter (e.g., K^+ , Na^+) in promoting the formation of SGQs, prompted us to evaluate their effects as well. Specifically, we evaluated the thermoresponsive behavior of **1–3** in the presence of the following iodide salts: LiI, NaI, and KI (all at 2 M). We recently reported the effects of a series of mono- and divalent cations on the structure and dynamics of SGQs in organic media.⁵⁵ The results of those studies indicate that varying the cation provides an efficient way to tune the molecularity, the fidelity, and the stability (thermal and kinetic) of the resulting SGQs. In the present study with these thermoresponsive SGQs, LiI does not seem to promote the formation of an SGQ (in a previous study in organic media⁵⁵ we showed that Li^+ promotes octameric SGQs of relatively low stability), although it did enhance the solubility of **1–3**. Similar to KI, however, NaI promotes the formation of thermoresponsive hexadecameric SGQs of **1–3** (Figure S7), where the differences in T_{cp} between both solutions are small or negligible (<2 °C, Figure 6). These findings are not surprising because both sodium and potassium cations are bound inside the SGQs, leading to similar supramolecular arrangements as we have reported recently.⁵⁵ Changes in the counteranions, on the other hand, should affect the surface of the SGQs, and we hypothesize that they would have a greater effect on the LCST, as described next.

Anion Effects

We evaluated representative anions from the Hofmeister series, specifically $H_2PO_4^-$, F^- , Cl^- , I^- , and SCN^- (as their corresponding potassium salts, all at 2 M with the exception of KF, which was used at ~0.9 M). Our results are consistent with the Hofmeister series, with kosmotropic anions ($H_2PO_4^-$, F^- , and Cl^-) preventing the dissolution of **1–3** and the most chaotropic anion SCN^- increasing the solubility of **1**₁₆, **2**₁₆, and **3**₁₆ so much that it abolished their LCST phenomenon for temperatures of up to 80 °C (Figure 6). These observations are consistent with multiple studies⁵⁶ that show the preferential distributions of large polarizable anions (e.g., SCN^-) on hydrophobic patches of amphiphilic macromolecules. We have recently shown that even subtle changes in the size and distribution of hydrophobic patches on the surface of the SGQs have significant effects on their corresponding LSCT.⁴⁴

But how do ions alter the LCST of macromolecules? The Hofmeister series, originally developed as a ranking of various salts according to their relative ability to promote the precipitation (salting-out) or solubilization (salting-in) of proteins,^{12,13} provides an empirical classification of electrolytes based on phenomena such as the properties of colloidal solutions.¹² In general, small ions of high charge density are strongly hydrated (kosmotropes) while large monovalent ions of low charge density are weakly hydrated (chaotropes).⁵⁷ These hydration differences lead in turn to different partitioning between the ions in solution and those interacting directly on the surface of the macromolecule.^{12,58,54} Considering that the LCST provides a rigorous measure of the hydrophobicity of a system,¹¹ which in turn reflects the interfacial phenomena between the dissolved macromolecule and the surrounding ions, it follows that the LCST will be sensitive to changes in dissolved salts.

pH Effects

We then evaluated the effect of pH changes on the LCST of **1**₁₆, **2**₁₆, and **3**₁₆ in the physiological pH range of 6–8. This was evaluated using four different phosphate-buffered solutions (prepared with KH₂PO₄/K₂HPO₄ containing 2 M KI) with target pH values of 6.0, 7.0, 7.4, and 8.0. Figure 7 shows the curves of T_{cp} as a function of temperature for **1**₁₆, **2**₁₆, and **3**₁₆ at different pH values. Under mildly acidic (pH 6.0), neutral (pH 7.0), and moderately basic (pH 8.0) conditions, the T_{cp} values corresponding to solutions of **1–3** (from three heating/cooling cycles) showed very little variation relative to experiments performed at pH 7.4. There is, however, a tendency toward slightly higher (1–2 °C) values of T_{cp} at pH 8 and a similar decrease in the values of T_{cp} at pH 6 (with the exception of **2**₁₆). This resistance toward relatively large pH changes underscores the robust nature of these SGQs and provides the necessary foundation for the future development of pH-responsive SGQs.

LCST Modulation via Supramolecular Parameters (Hetero SGQs)

Blending procedures and supramolecular modifications of polymeric systems have been used to modulate the LCST of responsive systems.⁵⁹ In our initial report on thermoresponsive SGQs, we also showed that making heteromeric SGQs (by mixing two SGQs with and without the LCST property) was an effective strategy to modulate the transition temperature.²⁴ We thus hypothesized that mixtures of **1**₁₆ (least hydrophobic, highest T_{cp}) and **3**₁₆ (most hydrophobic, lowest T_{cp}) would lead to a similar modulation of the T_{cp} , but in a range more suitable for potential biomedical applications. Turbidimetry measurements with the 1:1 mixture show T_{cp} values of around 40 °C, which are in fact intermediate between those of the corresponding homomeric SGQs (Figure 8, center). The results of the mixtures in the 1:3 and 3:1 proportions (Figure 8, top and bottom) are more complex, and although at first glance they may appear to be random, a closer inspection reveals a similar trend. The T_{cp} occurs in both cases at the lower end of the temperature range, steadily increasing (although not always with the same magnitude) toward the higher end of the temperature range. We hypothesize that this phenomenon results from an initial narcissistic self-sorting (i.e., preferential formation of homomeric SGQs)⁶⁰ where the lower T_{cp} corresponds to the most hydrophobic SGQ (**3**₁₆) in the mixture. Multiple heating/cooling cycles, however, may facilitate the formation of heteromeric SGQs whose effective hydrophobicity appears to be closer to the least hydrophobic SGQ (**1**₁₆) in the mixture. Although this mixing strategy provides a convenient method to modulate the LCST and

whereas ^1H NMR studies of the aforementioned mixtures (Figure S9) suggest that SGQs are indeed formed, the complexity of the spectra makes it difficult to determine with accuracy the exact proportions of the different supramolecular species (e.g., ratio of homomeric to heteromeric SGQs). Efforts to fully elucidate the structures and the mechanism of formation of these heteromeric SGQs (using isotopic labeling and mass spectrometry) are ongoing and will be published elsewhere.

CONCLUSIONS

We have designed and synthesized three mCGs that self-assemble into precise (nonpolymeric) thermoresponsive SGQs. Modulating intrinsic parameters, such as the group attached to the 8-*meta*-carbonyl moiety, enabled changes in the LCST of up to 30 °C. Adjusting extrinsic parameters leads to a broad range of variations in the LCST, going from negligible or small (e.g., replacing K^+ for Na^+ , changing the pH in the range of 6–8) to large (e.g., replacing I^- with SCN^-). Developing pH-responsive SGQs requires introducing into the monomeric subunit a functional element with a $\text{p}K_{\text{a}}$ value matching the pH range of interest, and efforts toward achieving this are ongoing in our group. Modulation of the LCST via changes in supramolecular parameters is feasible when mixing equimolar amounts of two different 8ArGs, but the behavior of mixtures in other proportions is complex and more studies are needed to fully understand it. These nonpolymeric SGQs provide an alternative strategy for the construction of a new class of thermoresponsive smart materials for biomedical and nanotechnological applications that can complement those of existing polymeric systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Cartoon depictions of the (a) isothermal self-assembly in aqueous media of hexadecameric SGQs: **1**₁₆, **2**₁₆, and **3**₁₆. (b) The thermoresponsive assembly of such SGQs (above the LCST) leads to the formation of SHS. The yellow and green circles represent cations and anions, respectively, and are included to underscore their potential role in modulating the properties of both the SGQs and the corresponding SHS. See ref 24 for a more detailed schematic representation of the thermally induced assembly used to form the SHS shown in b.

Figure 2.

(a) Partial 2D NOESY spectra showing signature cross-peak patterns of **1**₁₆, **2**₁₆, and **3**₁₆. (b) Partial ¹H NMR spectra showing the region of the NH and aromatic signals for **1**₁₆, **2**₁₆, and **3**₁₆ (500 MHz, 298 K; all at 10 mM, 10% D₂O in phosphate buffer solution, pH 7.4, 2 M KI). The red and blue cartoon represents the hexadecamers, and the red circles and blue triangles highlight selected signals for outer and inner tetrads, respectively.

Figure 3.

Cloud-point and DSC transition-temperature measurements. Changes in optical transmittance (measured at 500 nm) for the SGQs formed by **1** (top), **2** (center), and **3** (bottom) as a function of temperature (10 mM, 2 M KI, pH 7.4, potassium phosphate buffer). Error bars are expressed as the standard deviation, which are smaller than the size of the data points ($<\pm 0.1$). The DSC endotherms show two maxima corresponding to the T_M (left) and the T_m (right) (red and blue deconvolution peaks, respectively; the values for T are shown in Table 1).

Figure 4. Average hydrodynamic diameters (D_H) for the SHS formed by 1_{16} (top), 2_{16} (center), and 3_{16} (bottom) as a function of temperature as determined by DLS. (See the Supporting Information for detailed experimental conditions.)

Figure 5. Size distributions for the SGQs formed by **1**₁₆ (top), **2**₁₆ (center), **3**₁₆ (bottom), and the corresponding SHS, at selected temperatures, as determined by dynamic light scattering (DLS).

Figure 6.

Changes in optical transmittance (measured at 500 nm) as a function of temperature for aqueous solutions of SGQs 1_{16} , 2_{16} , and 3_{16} (10 mM) with KI, NaI, and KSCN salts (the error is ± 0.1 °C).

Figure 7.

Influence of the pH on the T_{cp} of 1_{16} (red), 2_{16} (green), and 3_{16} (orange) (all derivatives at 10 mM, 2 KI, measured at 500 nm) dissolved in potassium phosphate-buffer solution of varying pH values (6.0, 7.0, 7.4, and 8.0). The data shown results from three heating/cooling cycles with an error (standard deviation) of ± 0.1 °C.

Figure 8.

Cloud-point measurements for aqueous solutions of **1** and **3** mixed in the proportions shown (10 mM total combined concentration). All of the curves result from four consecutive heating/cooling cycles labeled, respectively, with the letters a–d (transmittance at 500 nm) (the error is ± 0.1 °C). The increase in transmittance above 60 °C is typical of these types of experiments and results from the sedimentation of the amorphous aggregates formed after the SGQs have melted.

Scheme 1.

Synthesis of 8-*meta*-Carbonyl-2'-deoxyguanosine (mCG) Derivatives 1–3

Table 1

Turbidimetry and Calorimetric Data for the SQQs Formed by 1, 2, and 3 As Determined by UV/Vis Spectrophotometry and DSC, Respectively

	T_{cp} (°C) ^a	T_M (°C)	T (°C) ^b	$H_{cal}(T_M)$ (kcal/mol)	T_m (°C)	$H_{cal}(T_m)$ (kcal/mol)
1₁₆	59	58.72 ± 0.08	0	4.60 ± 0.07	72.93 ± 0.09	4.72 ± 0.07
2₁₆	33	55.19 ± 0.06	22	12.4 ± 0.1	69.98 ± 0.05	15.5 ± 0.1
3₁₆	28	37.5 ± 0.1	10	0.85 ± 0.01	66.22 ± 0.02	5.47 ± 0.01

^aThe temperature at 50% transmittance was taken as the T_{cp} .

^b $T = T_M - T_{cp}$.