Enhancing the Mechanical Properties of Guanosine-Based Supramolecular Hydrogels with Guanosine-Containing Polymers

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Supporting Information

ABSTRACT: We demonstrate that multivalent, polymeric 8methoxyguanosine derivatives based on poly-(dimethylacrylamide) can enhance the mechanical properties of the low molecular weight hydrogelator 8-methoxy-2',3',5'tri-O-acetylguanosine at biologically relevant salt concentrations. It is proposed that these nongelling polymeric derivatives, under the conditions studied, can result in a significant enhancement of these supramolecular gels (e.g., for gels containing 1 wt % gelator G' can be increased from ca. 2000 Pa with no additive to 80 000 Pa) by acting as supramolecular cross-linking units. Two competing mecha-



nisms appear to play a role in these cogels. At low polymer concentrations the guanosine-containing polymers tend to act more as solubilizing agents for the gelator, thus weakening the gels, while at high guanosine-containing polymer concentrations the gels show a marked enhancement in mechanical properties consistent with them acting as supramolecular cross-linking agents. As such, the thermomechanical properties of these cogels depend on both the polymer: low molecular weight gelator ratio and the number of 8-methoxyguanosine repeat units present in the polymer additive. Thus, these polymeric guanosine-based additives impart the ability to tailor both the modulus and shear sensitivity of the gels. For example, cogels with a modulus ranging between ca. 95 and 80 000 Pa can be obtained through judicious selection of the type and amount of polymer additive.

INTRODUCTION

Supramolecular gels, formed by the self-assembly of low molecular weight gelators, show promise in numerous applications such as stimuli-responsive materials,¹ sensors,² biomaterials,³ liquid crystal materials,⁴ and personal care formulations.⁵ Generally, such materials self-assemble into long fibrillar structures that result in gelation of the liquid medium. The self-assembly of the gelator is controlled by weak and reversible noncovalent interactions, and as a consequence, these gels are susceptible to external stimuli, which can impart interesting mechanical properties such as temperature-sensitive reversible sol-gel formation, sheer sensitivity, and healing.⁶ However, it also means that most of these responsive gels are relatively weak, although recent reports have shown that nanocomposite supramolecular gels can exhibit enhanced mechanical properties while still retaining some of their responsive behavior.

One key aspect of these systems that is receiving attention is the design of a supramolecular gel that has specific targeted properties. Some success toward this goal has been achieved through chemically modifying gelators.⁸ An alternative approach involves utilizing multiple-component gels where their properties can be tailored by simply changing the ratio of the different components.⁹ For a two-component system there

are a variety of different classes of such gels: (1) the two compounds form a gel only in combination, 10^{10} (2) both compounds are gelators and either (a) interact with each other to form cofibers¹¹ or (b) self-sort resulting in two different nanofibers within the gel,¹² and (3) gelator plus a nongelling additive component that is designed to impact the gel's thermomechanical or functional properties.¹³ In prior studies we have focused on this latter two-component system by designing nongelling additives and investigating how they can be used to impact the properties of the supramolecular gel. For example, we and others¹⁴ have shown that surfactants added to certain gelators can be used to help inhibit or prevent crystallization of a gelator. In particular, we have recently shown that the nonionic surfactant, Laureth-4, can result in long-lasting hydrogels of an amphiphilic glucose-based gelator and that both shear modulus and yield stress can be altered by controlling the ratio of the two components.¹⁵

Another class of gelator that we have previously investigated in two-component systems is guanosine (G) and its derivatives, which are well-known hydrogelators.¹⁶ In the presence of metal

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Figure 1. (a) (i) G-quartet formed around M^+ ions and (ii) a continuous G-helix formed around Na⁺ ions. (b) Schematic of guanosine-containing helical assemblies formed by **80MeTAcG** in the presence of Na⁺ ions with (left) a polymerizable G-derivative (1) and (right) with a guanosine-containing polymer (2 or 3).

ions (such as K⁺) they generally self-assemble in to G-quartets (Figure 1ai), in which the guanine units bind to the metal ion on the inside of the stack and the ribose units are on the surface. Subsequently, these quartets assemble into octamers and then into one-dimensional quadraplexes that eventually yield percolating assemblies resulting in gel formation.¹⁷ Using the cogel approach, we have shown that mixing G with a nongelating, more hydrophobic guanosine derivative (2',3',5'tri-O-acetylguanosine, TAcG) results in the formation of stable gels in aqueous potassium chloride (0.354 M).¹⁸ Here the guanine moiety in TAcG allows it to be incorporated into the stack, and as such the surface of the quadraplex consists of both ribose and triacetylribose units. By varying the ratio of the two components, the thermomechanical properties of the gels can be systematically altered. The data suggested that increasing the ratio of the larger, more hydrophobic triacetylribose units resulted in a shortening of the fibers (presumably on account of steric congestion) as well as stronger fiber-fiber interactions at slightly elevated temperatures (ca. 40 °C) on account of the increased fiber surface hydrophobicity. A similar approach has also been shown to work with other guanosine derivatives.¹⁹ For example, we recently reported²⁰ a new guanosine hydrogelator, 8-methoxy-2',3',5'-tri-O-acetylguanosine (80Me-TAcG), that forms gels at biologically relevant salt concentrations (ca. 100 mM NaCl, 5 mM KCl) and cell media, something that is not possible with guanosine.²¹ In this case the data suggests that rather than forming G-quartet stacks this

gelator self-assembles with Na⁺ ions into a continuous guanosine helix (Figure 1aii). Nonetheless, the mechanical properties of these gels can still be tailored with the addition of **TAcG**. Specifically, the modulus and yield stress can be reduced by the addition of **TAcG** by around 1-2 orders of magnitude at room temperature.

In all the cases detailed above adding in the nongelling additive results in a decrease in the mechanical properties of the supramolecular gel at room temperature. Thus, it became of interest to see if additives could be found that would enhance the room temperature mechanical properties of the gels. Figure 1b shows an idealistic schematic of one possible way to achieve this. The concept here is to use polymeric multivalent guanosine derivatives as potential supramolecular cross-linkers for the 8-methoxyguanosine assemblies. In particular, reported herein is the synthesis of polymerizable guanosine derivatives (1 and 2), their copolymerization with dimethyl acrylamide to yield a series of water-soluble, low molecular weight polymers (3 or 4), and an investigation of the effect that these "cross-linking" polymers have on their cogels with 8OMeTAcG at biologically relevant Na⁺ salt concentrations (100 mM).

The addition of a polymeric additive to low molecular weight gelators has not received much attention in the literature. However, there are reports by a few of groups on how polymers may impact the properties of a supramolecular gel.²² Some early work by Hanabusa showed that selected commercial polymers could be used to enhance the mechanical properties of an organogelator,²³ although no mechanism was proposed for this enhancement. There are examples of systems in which specific interactions, e.g. electrostatic,²⁴ nucleobase,²⁵ hydrogen bonding,²⁶ have been designed between the polymer and gelator. In such cases a number of different mechanisms have been proposed for how the polymer affects the gels thermomechanical properties, including the polymer impacting the supramolecular assembly and/or the crystallization of the gelator. Other researchers have investigated the effect of mixing polymeric gelators and low molecular weight gelators.²⁷ The goal of this specific study was to focus on polymers that have side chains which are derived from the low molecular weight gelator itself and as such can be directly incorporated into the gel-forming supramolecular assembly.

RESULTS AND DISCUSSION

There are a few reports of guanosine derivatives being incorporated into synthetic linear polymers.²⁸ Our approach, which targeted the synthesis of the guanosine-containing monomers (1 and 2) and their corresponding copolymers (3 and 4), is shown in Figure 2. Starting with the known 2'-3'isopropylidene guanosine-5'-carboxylic acid²⁹ (5) (prepared in two steps from guanosine (G) in 40% overall yield) the 2'- and 3'-positions were deprotected with 1 M HCl³⁰ to yield (98%) guanosine-5'-carboxylic acid (6). The 8-position of the guanine moiety was then functionalized with bromine³¹ (7, 80% yield) before being converted to a methoxy group with sodium methoxide to yield 8 (65%).³⁰ The 2'- and 3'-positions were then functionalized with acetyl groups using acetic anhydride DMAP and triethylamine (45% yield).³² Finally, the 5'-position was reacted with 2,2'-(ethylenedioxy)bis(ethylamine)monoacrylamide, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBop), and diisopropylethylamine (DIPEA) to yield the target polymerizable 8methoxyguanosine derivative, 1 (80%). The polymerizable guanosine derivative 2, which does not have the 8-methoxy



Figure 2. Scheme of polymerizable guanosine derivatives. Conditions: (a) 70% HClO₄, acetone, 3 h;³³ (b) NaHCO₃, TEMPO, iodobenzene diacetate, MeCN/H₂O, 3 h; (c) 1 N HCl, 65 °C, 1 h; (d) Br₂, H₂O, 1 h; (e) NaOCH₃, DMSO, MeOH, 65 °C, 18 h; (f) (CH₃CO)₂O, MeCN, TEA, DMAP, 4 h; (g) 2,2'-(ethylenedioxy)bis(ethylamine)-monoacrylamide, PyBop, DIPEA, DMF, 18 h; (h) 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid, DMA, AIBN, DMF, 70 °C, 10 h.

substituent, was prepared using similar chemistry (via acetylation and peptide coupling of 6) in 17% overall yield starting from **G** (Figure 2).

Cogels of 80MeTAG with the Monomer 1 or 2. Initial studies were carried out to determine if the acrylamide-functionalized guanosine monomers (1 and 2) were able to form gels with **80MeTAcG**. Mixtures of **80MeTAcG** and 1 or 2 were made in varying ratios (10:0, 7:3, 5:5, 3:7, 0:10) in 100 mM NaCl where the total content of the two compounds was set at 2 wt % (Figure 3a). Gratifyingly, within 15 min stable gels could be formed under these conditions at **80MeTAcG**:1 (or 2) ratios ranging from 10:0 to 3:7. Neither 1 nor 2 formed a gel by itself, most likely on account of their enhanced, relative to **80MeTAcG**, water solubility. To confirm that 1 and 2 can be incorporated into the **80MeTAcG** assemblies, variable temper-

5:5 5:5 a) 80MeTAcG: 1 80MeTAcG: 2 b) 10[€] 10 : 0 80MeTAcG-1 3 8OMeTAcG: 7 5 8OMeTAcG:1 10 G' (Pa) 10 10² 100 0.1 10 1000 τ (Pa) C) 10[€] 10: 0 80MeTAcG:2 3 8OMeTAcG:2 5 8OMeTAcG:2 10 (Pa) ΰ 10^{3} 10² 0.1 10 100 1000 τ (Pa) d) 10^t 1:0 80MeTAcG:1 . 1:0.2 8OMeTAcG:1 1:0.5 80MeTAcG: 10 (Pa) ö 104 0.1 1 10 100 1000 τ (Pa)

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Figure 3. (a) Images of 2 wt % 5:5 **80MeTAcG:1** and 2 cogels in 100 mM NaCl; stress sweep experiments ($\omega = 6.28 \text{ rad/s}^{-1}$, $T = 25 ^{\circ}$ C) of 2 wt % 100 mM NaCl gels of (b) **80MeTAcG:1**, (c) **80MeTAcG:2**; and (d) **80MeTAcG:1** gels with varying wt % of 1 while holding **80MeTAcG** concentration at 1 wt %.

ature NMR (VT-NMR) studies were undertaken; NMR will show the guanosine derivatives in the solution phase of the sample but not in the gel phase.^{20,34–36} In these experiments the NMR of a 1:1 mixture of the two components is taken as the sample is cooled from 75 to 25 °C and the percentage of the components in the sol determined by integration of their acetyl protons. 75 °C is well above the gel–sol transition temperature ($T_{gel-sol}$) of **8OMeTAcG** (ca. 63 °C) and is a temperature where the ratio of the two components in the solution is measured to be 1:1 by NMR. These variable temperature experiments suggest that 69% of 1 and 78% of

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80MeTAcG are incorporated into the **80MeTAcG**:1 gel phase at room temperature. 2 is incorporated to a lesser degree (62%) in the **80MeTAcG**:2 cogels with 80% **80MeTAcG** being present in the gel phase (Figures S1 and S2). It is important to note that guanosine derivatives tend to form stacks of G-quartets while, from our previous work,²⁰ the **80MeTAcG** prefers to form continuous helical assemblies on account of the presence of both the methoxy moiety and the bulky acetyl groups. Thus, it is reasonable that 1 (with its 8-methoxy substituent) is more easily incorporated into the helical assembles of **80MeTAcG** than **2**.

Stress sweeps (from 0.1 to 1000 Pa and a frequency of 6.28 rad/s) were performed on all cogels at 25 °C. As was the case with our previous studies,^{18,20} increasing the amount of the nongelator (1 or 2 in this case) results in a systematic decrease in the mechanical properties of the cogel (Figure 3a–c, Figures S3 and S4). It is worthy of note that the G' of the 1 cogels (i.e., 6000 Pa for 7:3) is significantly greater than the 2 cogels (i.e., 670 Pa for 7:3), consistent with 1 being incorporated better/ more strongly (relative 2) within the **80MeTAcG** gelator assemblies.

It is important to mention that in the above studies increasing the amount of 1 or 2 decreases the amount of gelator, **80MeTAcG** (total wt % was held at 2%), which is presumably also contributing to the decrease in G'. Therefore, similar stress sweep experiments were performed on cogels of **80MeTAcG**:1 that kept the concentration of the gelator **80MeTAcG** at 1 wt % but varied in the amount of 1 (Figure 3d). Again, a drop in G' is seen with an increase in 1; however, almost no difference in the yield points is observed.

Synthesis of Guanosine-Containing Copolymers 3 and 4 and Their Cogels with 80MeTAG. Both 1 and 2 were copolymerized with the water-soluble monomer dimethylacrylamide (DMA) via RAFT polymerization to yield 3 and 4, respectively (Figure 2). All copolymers were targeted with a degree of polymerization (DP) of ca. 30. 1 was copolymerized with DMA at three different DMA:1 ratios-25:5, 20:10, and 15:15-while 2 was polymerized with DMA in a ratio (DMA:2) of 25:5. The three copolymerizations of 1 and DMA yielded polymers with different ratios of the two repeat units (DMA:1) but similar DPs; 3_4 (21:4, $M_n = 4500$, DP = 25), 3_7 (18:7, $M_n = 6000$, DP = 25), and 3_{10} (15:10, $M_n = 7400$, DP = 25) (see Table S1). The copolymerization of DMA and 2 yielded a polymer (4_4) with a molecular weight of 4300 g/mol (DP = 25) and a ratio of 21:4 (DMA:2 repeat units). The homopolymer of poly(dimethylacrylamide) $3_0 (M_n = 2800 \text{ g/}$ mol, DP = 28, D = 1.15) was also synthesized in the similar manner to the guanosine-containing polymers as a control.

Cogels were made in 100 mM aqueous NaCl keeping the amount of **80MeTAcG** (1 wt %) the same and varying the ratio of 3_x or 4_4 (1:0.25, 1:0.5, 1:1, and 1:1.5, based on molar equivalents of **80MeTAcG** to the repeat units of **1** or **2** in the polymer; see Table S2 for wt % details). For example, for the 1:0.25 **80MeTAcG**: 3_4 system the amount of 3_4 was added such that the polymer 8-methoxyguaninosine repeat units were 0.25 mol equiv of **80MeTAcG**. For the 3_0 cogel the same gram amount of polymer was added to the system as with the 3_4 cogel. It is important to note that these cogels do not form immediately upon initial mixing but do form upon standing for a period of time. For example, the 3_4 comixtures gelled (as determined by the upside down test) within 2–3 h and the 4_4 cogels formed in 5–6 h, while the 3_7 and 3_{10} comixtures took 12–14 h to form the gels. Furthermore, none of the polymers

formed gels by themselves in 100 mM aqueous NaCl, even at high concentrations (up to 50 wt %), which is presumably related to their good water solubility.

VT-NMR experiments were performed on 1:1 samples of **80MeTAcG** and 4_4 and 3_4 to determine the extent of incorporation of each component into the gel phase (Figures S5 and S6). It was found that in 8OMeTAcG:34 ca. 82% of 8OMeTAcG and 74% of the 8-methoxyguanosine repeat units from 3_4 are incorporated into the gel phase, while in 8OMeTAcG:44 about 79% of 8OMeTAcG and 68% of the guanosine repeat units from 4_4 are incorporated. Thus, the incorporation of the polymeric guanosine derivatives $(3_4$ and 4_4) and the amount of the gelator in the gel phase, at the 1:1 ratio, are similar to those of their respective monomers (1 or 2). The thermal properties of the 8OMeTAcG and the 1:1 **80MeTAcG:1**, 2, $3_{x'}$ and 4_4 gels were analyzed by differential scanning calorimetry (DSC) (Figure 4). The DSC for each gel shows a peak that is consistent with the $T_{\rm gel-sol}$ which for the gelator 80MeTAcG itself is ca. 63 °C. Incorporation of monomer 1 into the gel does not change the $T_{gel-sol}$ temperature (63 °C). However, addition of homo-polyDMA (3_0) does result in a significant drop in the $T_{\text{gel-sol}}$ (to ca. 51 °C), suggesting that this polymer has a significant destabilizing effect on the gel. For the 8-methoxyguanosine-containing polymer additives $\mathbf{3}_{\mathbf{x}}$ there is a systematic increase in the $T_{\text{gel-solv}}$ i.e. $\mathbf{3}_4$ (~62.5 °C), $\mathbf{3}_7$ (~65 °C), and $\mathbf{3}_{10}$ (~67 °C), consistent with an increase in the stability of the gel with an increase in the number of these binding units in the backbone. The $T_{\rm gel-sol}$ of the **8OMeTAcG** cogels with the guanosine derivatives; $2(\sim 56)$ °C) and the copolymer 4_4 (~58 °C) are significantly lower than any of the 8-methoxyguanosine derivatives and only slightly higher than 3_0 , which is consistent with this guanine moiety not being as effectively bound within the 8OMeTAcG assemblies as the 8-methoxyguanosine units.



Figure 4. DSC of 2 wt % 80MeTAcG and 1:1 80MeTAcG: (1, 2, 3₄, 3₇, 3₁₀, and 4₄) cogels in 100 mM NaCl.

Circular dichroism measurements were performed on 0.3 wt % 80MeTAcG, 1:1 80MeTAcG:1, and 1:1 80MeTAcG:3₄ gels in 100 mM NaCl (Figure 5a) to better understand the nature of their assembly.³⁷ The CD spectrum for 80MeTAcG:1 and 80MeTAcG:3₄ are similar to that of 80MeTAcG alone with diagnostic peaks at ca. 280, 250, and 210 nm. The peak at 280 nm is evidence for a tilting or deformation of the tetrameric G planes, consistent with the formation of continuous G-helix assemblies rather than G-quartet formation.^{38,39} To obtain further insight into the assembly of the gels, transmission electron microscopy (TEM) was performed.



Figure 5. (a) CD spectra of 1 wt % 80MeTAcG, 1:1 80MeTAcG:1, and 1:1 8OMeTAcG:34 in 100 mM NaCl. TEM images of (b) 80MeTAcG and (c) 80MeTAcG:34 in 100 mM NaCl.

Images of 8OMeTAcG and 8OMeTAcG:310 (Figure 5b,c) show that bundles of nanofibers are present within both gels. TEM images of 8OMeTAcG:2 and 8OMeTAcG:44 can be seen in Figure S7 and also show the presence of fiber bundles. A comparison of these images does suggest the formation of a greater number of larger bundles in the polymer-containing gels, although the evidence here is not definitive. AFM height profiles indicate that the average height for the single fibers in the 1:1 8OMeTAcG:1 gel is 2.97 ± 0.82 and 3.14 ± 0.52 nm for the 1:1 8OMeTAcG:3₄ gel (Figure S8).

Figure 6 shows a comparison of the stress sweep experiments of all the 1:1 3x:80MeTAcG cogels along with the 8OMeTAcG gel. Here the mechanical properties of the gel varies from the 30:80MeTAcG gel (268 Pa) to the 310:80MeTAcG gel (35 000 Pa) This data clearly shows that for the 1:1 cogels (1) the poly(dimethylacrylamide) (3_0) negatively impacts the mechanical properties of the gels and (2)increasing the amount of guanosine repeat units results in greater mechanical enhancement of the gel. Thus, the rheology data matches the $T_{\text{gel-sol}}$ data obtained from the DSC experiments (vide supra) and is consistent with the 8-



10

10

(Pa)

Figure 6. Comparisons of stress sweeps of 1:1 80MeTAcG cogels with 1, 30, 34, 37, and 310 in 100 mM NaCl at 25 $^\circ\text{C}.$

τ (Pa)

methoxyguanosine units binding into the helical 8OMeTAcG assemblies and the polymers acting in some way as crosslinking units. A key aspect that appears to play a role in these systems is the 8OMeTAcG gel/sol content of the gels. NMR data (see Figure S9) show that the amount of 8OMeTAcG incorporated into the gel content increases, from ca. 68% for the 3_0 cogel to 87% for the 3_{10} cogel, with the number of 8methoxyguanosine units in the polymer backbone.

Rheology studies at different gelator:polymer ratios (Figures S10-12) indicate a more complicated behavior than suggested by Figure 6. While the 1:1.5 **8OMeTAcG**:3_x gels show a similar trend to the 1:1 cogels, the 1:0.25 show a completely different behavior. In these samples the strongest cogel is formed with the polymer with the fewest number of 8-methoxyguanosine repeat units (3_4) . NMR data (Figure S9) suggest that at these lower concentrations this may be a result of the polymers 3_7 and 3_{10} aiding the solubility the 8OMeTAcG gelator; the amount of **80MeTAcG** present in the sol phase of the 3_4 , 3_7 , and 310 1:0.25 cogels is 12, 22, and 21%, respectively.

Figure 7a-d shows the data for the cogels of 3_4 , 4_4 , 3_7 , and 3_{10} , respectively, at the different gelator to polymer ratios and highlights a number of other interesting points. One of the first things to note is that the mechanical enhancement effect of 3_4 (Figure 7a) is greater than 4_4 (Figure 7b) at all ratios, e.g. 11 000 Pa versus 4000 Pa at a ratio of 1:0.25. This is again consistent with the previous data suggesting 8-methoxyguanosine units are incorporated more efficiently in to the helical 80MeTAcG assemblies. However, both follow the trend of a reduction in the gels' storage modulus and yield stress as the concentration of polymer increases. Interestingly, a very different behavior is observed for the two polymers that have more 8-methoxyguanosine repeat units within their backbone $(3_7 \text{ and } 3_{10})$. In the 8OMeTAcG: 3_{10} cogel, both G' and yield stress increase with the concentration of the polymer additive (Figure 7d), while the **8OMeTAcG**:37 cogel exhibits a behavior in-between where the maximum modulus (8500 Pa) and yield stress (142 Pa) are obtained at a 1:1 ratio of 80MeTAcG:37 (Figure 7c).

Thus, it appears that both the ratio of **8OMeTAcG** to the 8methoxyguanosine repeat units as well as the ratio of 80MeTAcG to polymer is important in determining the mechanical properties of these cogels. Generally, higher mechanical properties are observed either with high concentrations of polymers with a large number 8-methoxyguanosine repeat units or with low concentrations of polymers with a low number 8-methoxyguanosine repeat units. This interesting behavior can be rationalized by understanding how much of the **8OMeTAcG** is incorporated in to the gel phase. For example,



Figure 7. Comparisons of stress sweeps ($\omega = 6.28 \text{ rad/s}^{-1}$, T = 25 °C) of 1:1 80MeTAcG cogels with 1, 3₀, 3₄, 3₇, and 3₁₀ and 1 wt % 80MeTAcG in 100 mM NaCl at 25 °C. (a) Stress sweep experiments of 80MeTAcG and (a) 3₄, (b) 4₄, (c) 3₁₀, and (d) 3₇ gels in 100 mM NaCl.

at a ratio of 1:0.25 the amount of 8OMeTAcG incorporated in the gel phase decreases upon increasing the number of 8methoxyguanosine repeat units in the polymer, e.g. 88% for the 3_4 gel and 79% for the 3_{10} gel. However, the opposite trend is observed at much higher polymer concentrations, e.g. at a 1:1.5 80MeTAcG:3, ratio 84% of 80MeTAcG is incorporated in the gel phase of the 3_4 gel while 89% is incorporated in the 3_{10} gel (Table S2). It is important to note that even though the gel content is similar in the 1:0.25 3_4 cogel and the 1:1.5 3_{10} cogel their mechanical properties, 11 000 vs 80 000 Pa, respectively, are very different. Thus, there appears to be two main factors that impact the properties of the gel. At low polymer concentrations a key factor appears to be the solubility of the 8OMeTAcG with the polymer with a larger number of 8methoxyguanosine side chains, resulting in higher solubility of the gelator and thus weaker mechanical properties. As the concentration of the polymer is increased, then the mechanical properties are enhanced by the presence of more 8methoxyguanosine repeat units in the polymer, consistent with supramolecular cross-linking occurring at the higher polymer concentrations.

Frequency-sweep experiments were carried on 1 wt % of the **8OMeTAcG** gel and 1:1 **8OMeTAcG** cogels with 3_0 , 3_4 , 3_7 , 3_{10} , and 4_4 to further examine their properties (Figure 8). The data indicate that for the **8OMeTAcG** gel and all **8OMeTAcG**: 3_x cogels the moduli are independent of frequency over the entire sweep range, with G' > G'' at all points. Interestingly, however, the 3_0 and 4_4 cogels behave very differently with G' and G'' approaching similar values at low frequency (long time scales). This behavior indicates that in these cogels the entangled fiber assembles can disentangle/ disengage at long time scales, resulting in much weaker gel



Figure 8. Frequency sweeps of (a) 2 wt % **80MeTAcG** gel and 1:1 **80MeTAcG**:3₀, 3₄, 3₇, and 3₁₀ gels in 100 mM NaCl; (b) 2 wt % 1:1 **80MeTAcG**:3₄ and 4₄ gels in 100 mM NaCl. T = 25 °C, $\gamma = 0.2\%$.

characteristics.⁴⁰ The most interesting comparison to be made here is between the cogels of 3_4 and 4_4 (Figure 8b), which shows the significant weakening effect that removal of the 8-

methoxy substituent on the polymeric guanosine moiety has on the properties of the cogels. The fact that **8OMeTAcG**: $\mathbf{3}_4$ is a strong gel at all the frequencies studied while the **8OMeTAcG**: $\mathbf{4}_4$ gel exhibits significant weakening at longer time scales is consistent with the 8-methoxyguanosine repeat units in $\mathbf{3}_4$ being bound more tightly into the **8OMeTAcG** fiber network than the guanosine repeat units in $\mathbf{4}_4$.

One key property of supramolecular gels is their shear sensitivity which allows them, for example, to be injectable. A key property of any injectable gel is its ability to reform after the injection. Shear recovery experiments were therefore performed on 1:1 cogels (along with the 2 wt % 80MeTAcG gel) to examine how long it takes these gels to recover after being exposed to shear. Gels were monitored at 0.1 Pa for 2 min, a stress of 250 Pa was applied for 30 s to force the gels to vield, and then the gels were monitored at a stress of 0.1 Pa for 15 min to measure their recovery time (Figure 9a). The data indicate that while for all the gels G' becomes greater than G''by 50 s, full recovery of the mechanical strength of the gels does take longer with the polymer additives and is dependent on the number and type of the guanosine repeat units. For example, the 2 wt % 8OMeTAcG gel fully recovers within 50 s, while the 1:1 8OMeTAcG:34 gel recovers in ca. 69 s, the 1:1 80MeTAcG:47 gel at ca. 97 s, and a 1:1 80MeTAcG:310 gel in ca. 310 s. This difference in behavior can be visualized in Figure 9b which shows images of 8OMeTAcG and 1:1.5 **80MeTAcG:3**₁₀ (chosen as it has the highest G' value) gels that have been injected through an 18-gauge needle and onto a glass slide. While both gels can be injected and both re-form the images clearly show that the 80MeTAcG gel forms immediately after being injected, while the 3_{10} gel is more spread out on the slide consistent with it taking a little longer to fully recover.



Figure 9. (a) Shear recovery of 2 wt % 80MeTAcG and 1:1 gels of 80MeTAcG:3_x (1:1 based on molar ratio) in 100 mM NaCl. Gels were monitored at 0.1 Pa for 2 min, subjected to stress of 250 Pa to yield, and recovery was monitored at 0.1 Pa for 15 min. ω = 6.28 rad/s, T = 25 °C. (b) Images of a droplet of 80MeTAcG and a 1:1.5 80MeTAcG:3₁₀ gel on a glass slide 30 s after being injected through a 18-gauge needle.

CONCLUSIONS

We have synthesized a new series of 8-methoxyguanosine/ dimethylacrylamide (DMA) copolymers and shown that addition of these nongelling copolymers to the low molecular weight gelator 8OMeTAcG results in significant changes to the mechanical properties of the supramolecular hydrogel. The data show that the 8-methoxyguanosine repeat units in the polymer backbone are incorporated into the helical assemblies formed by the gelator, suggesting the possibility of the polymers acting as supramolecular cross-linking units. In addition, the polymers also impact the solubility of the low molecular weight gelator (8OMeTAcG) which, particularly at lower polymer concentrations, can negatively impact the properties of the gels. The mechanical properties of the cogels were shown to be related to a number of factors, such as the ratio of the two components (gelator to polymer), the ratio of the polymeric 8methoxyguanosine and DMA repeat units (relative to the gelator), and the chemical structure of the guanosine derivative on the polymer. Thus, by judicious choice of the previously mentioned factors the modulus of the 1 wt % 80MeTAcG hydrogel (2000 Pa) can be varied from 95 to 80 000 Pa (at similar gelator concentration) through simple addition of the nongelling polymer. Furthermore, all these gels are shear sensitive. Exposure to shear results in a free following liquid which re-forms the gel relatively quickly upon removal of this stimulus, allowing these gels to be injectable.

ASSOCIATED CONTENT

S Supporting Information

Experimental section including VT-NMRs, TEM images, polymerization data and compositions, rheology data, AFM, NMR, and MALDI of guanosine derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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